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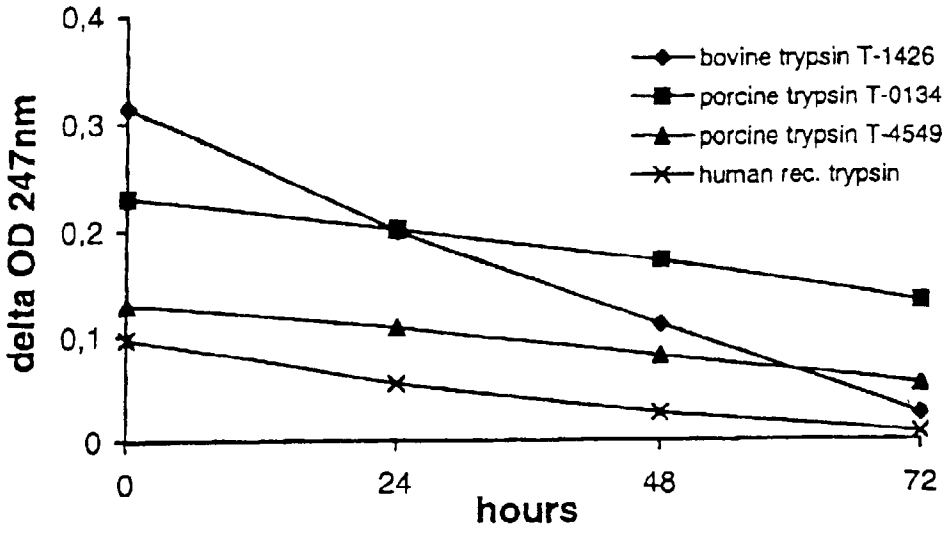


(57) Abstract: The invention relates to a simple and efficient process for isolating viruses from various sources and for producing live attenuated influenza vaccines in a serum-free Vero cell culture under conditions where alterations in the surface antigens of the virus due to adpative selection are minimized or prevented. The process does not require purification of the virus-containing supernatant harvested from the cell culture nor post-incubation treatment of the viruses for HA activation. The invention further relates to influenza A and B master strain candidates and to vaccines made thereof.

WO 02/24876 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

LIVE VACCINE AND METHOD OF MANUFACTURE

TECHNICAL FIELD

The present invention is in the field of virology and vaccine development and relates to an improved method of manufacture of a viral vaccine, particularly of a whole-virus vaccine, preferably of an attenuated live vaccine and to vaccines obtainable by the method.

BACKGROUND OF THE INVENTION

The influenza hemagglutinin (HA) antigen is the major target for the protective immune responses of a host to the virus.

A common practice of recovering new viral isolates involves recovery from a nasal or throat swab or from a similar source, followed by cultivation of the isolates in embryonated chicken eggs. The virus adapts to its egg host and large scale production of the virus can be carried out in eggs. Such conventional methodology involving embryonated chicken eggs to produce Influenza vaccine is, however, extremely cumbersome, involving the handling of many thousands of eggs per week as well as extensive purification of the virus suspension derived from the allantoic fluid to ensure freedom from egg protein.

Another disadvantage in the use of chicken embryos for virus production lies in the fact that this substrate strongly favors the selection of virus variants that differ in their antigenic specificity from the wildtype virus and not rarely results in viruses that may not be suitable for vaccine production due to their altered phenotypes including, for instance, considerable reduction in immunogenicity.

Many attempts have therefore been undertaken in the art to utilize standard tissue culture technology with established mammalian cell lines, such as MDCK (Madin-Darby Canine Kidney) or Vero (African Green Monkey Kidney) cells, for virus production, particularly influenza virus production.

One of the difficulties in growing influenza strains in tissue cell culture arises from the necessity for proteolytic cleavage of the influenza hemagglutinin in the host cell. Cleavage of the virus HA precursor into the HA1 and HA2 subfragments, although not necessary for the assembly of the viral elements to

form a complete virion, is required, however, to render the virion infective, i.e. to enable it to infect a new cell.

It has been reported (e.g. Lazarowitz et al., "Enhancement of the Infectivity of Influenza and B Viruses by Proteolytic Cleavage of the Hemagglutinin Polypeptide", Virology, 68:440-454, 1975) that the limited replication of several influenza A strains in standard cell cultures could be overcome by the addition of proteases like trypsin to the tissue culture medium. Yet, there remained difficulties in some cases, for instance when using Vero cells.

Kaverian and Webster (J Virol 69/4:2700-2703, 1995) report that in Vero cell cultures, and less pronounced in MDCK, swine kidney, or rhesus monkey kidney cell cultures, the trypsin activity in the medium rapidly decreased from the onset of incubation resulting in the failure of virus accumulation in the medium due to the lack of production of a sufficient number of infective virions. They concluded that a trypsin inhibiting factor was released from the Vero cells. They further showed that by repeated addition of trypsin reproduction of virus could be resumed and maintained for a number of reproduction cycles resulting in a much better virus yield.

Another way for efficient vaccine production was reported in US 5,753,489 wherein serum-free medium was used for virus propagation in a number of different mammalian cells including MDCK and Vero cells. The method disclosed therein comprises growing vertebrate cells in serum-free medium, infecting the cell culture with a virus, incubating the cell culture infected with the virus, removing a portion of the virus-containing medium and contacting this portion with a protease, thereafter adding to that portion a protease inhibitor and returning that portion to the cell culture. It is preferred therein to provide the steps of growing, infecting and incubating in a first vessel and the steps of trypsin-contacting and inhibitor-adding are performed in a second vessel connected with the first vessel in a loop so that the steps o can be performed in a closed cycle. This system allows to use trypsin or other proteolytic enzymes at much higher concentrations than those normally tolerated by cells in culture.

EP 0870508 reports a method to produce a viral antigen vaccine comprising infecting an animal cell line, optionally a Vero cell line, with virus, propagating virus in the cell culture, adding a nuclease enzyme to the cell culture shortly

before the end of virus propagation to digest nucleic acid material released from the lysing host cells into the medium, harvesting the virus and obtaining viral antigens thereof by extraction in order to make the viral antigen vaccine. The patent is silent with regard to the kind of nutrient medium used for virus propagation and also with regard to the addition of a protease, usually required for the final processing of influenza virus hemagglutinin to get infectious virus. The method further requires various purification steps for providing a ready-for-use vaccine preparation.

It is known, however, that the nature the host substrate as well as the composition of the nutrient medium used for virus propagation may significantly affect immunogenicity and antigenicity of the virus progeny obtained therewith. Particularly, serum-containing media may not only decrease antigenicity of viral progeny but additionally may decrease protease activity in the medium, hence inhibit virus maturation, and subsequently require expensive steps of purification.

SUMMARY OF THE INVENTION

The present invention overcomes the drawbacks of the prior art. It relates to a simple and efficient process for isolating viruses from various sources and for producing viral progeny for use as vaccines, particularly live attenuated influenza vaccines, in under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or entirely prevented.

It is also an object of the present invention to provide for a method for the production of viruses, particularly influenza viruses, that yields viral progeny that selectively agglutinates human erythrocytes but not chicken erythrocytes, and that preferably has antigenic properties identical with those of the initially inoculated virus strain, e.g. a primary clinical wildtype isolate.

In a preferred embodiment, the nucleic acid sequence of the HA gene and optionally of the NA gene of the propagated virus is identical with the one of the initially inoculated strain (e.g. an epidemic strain, primary clinical isolate of an infected patient).

It is another object of the invention to provide a method for efficient production of a whole-virus vaccine, particularly a live attenuated vaccine, in a single step procedure that does not require any chromatographic or other purification steps of the virus suspension harvested from the cell culture supernatant by centrifugation, particularly no protein separation or purification steps.

It is yet another object of the invention to provide attenuated, cold adapted and temperature sensitive influenza A and B strains and vaccines made thereof.

BIREF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a Vero cell culture.

Fig. 2 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a MDCK cell culture.

DETAILED DESCRIPTION OF THE INVENTION

Comparative experiments using embryonated eggs, MDCK and Vero cells clearly proved that the initially inoculated virus is likely to undergoe antigenic alteration during growth on any one of these substrates

Our experiments confirmed that the alterations are least or even absent for influenza virus strains grown on Vero cells in serum-free medium. Moreover, it turned out that influenza A viruses, at least strains of the H3N2 subtype, when multiplied on Vero cells in serum-free and protein-free medium exhibit a selectivity for agglutination of human erythrocytes but not for chicken erythrocytes. Also, they did not grow on eggs. This was a first indication that these Vero-grown viruses might be more identical with the wildtype virus of the corresponding clinical isolate than the ones grown on MDCK cells or eggs.

Indeed, comparison of the HA and NA gene sequences of wildtype isolates obtained from nasal swabs with the ones of the same viruses after growth on Vero and MDCK cells, respectively, revealed alterations in the HA or NA of MDCK-grown viruses relative to the HA or NA of the swab isolates or of the Vero-grown viruses or of both the swab isolates and the Vero-grown viruses.

Moreover, experimental data obtained from immunizations of ferrets with Vero- and MDCK-grown wildtype viruses indicate a far stronger virulence of the Vero-grown viruses compared to the MDCK-grown viruses. Also, the immunogenicity of the Vero-grown viruses tested in an animal trial on macaques was demonstrated to be significantly superior to the one of the viruses grown on MDCK cells or eggs.

These findings together provide strong evidence for the hypothesis that the process for the multiplication and propagation of viruses according to the present invention as hereinafter described in more detail yields viruses that are either unaltered compared to the initially inoculated (e.g. wildtype) virus or are modified to only a minor extent.

It is not only the avoidance of antigenic alterations that makes the present process of virus multiplication so unique, but it is also its striking simplicity which makes it extremely suitable for large scale industrial vaccine production.

Further experiments have shown that the source of trypsin (or trypsinogen) may be one additional factor that influences the overall yield of infective virions. Indeed, while the methods known in the art (e.g, Kaverin and Webster, J Virol 69/4:2700-2703, 1995; or US 5,753,489) use either repeated addition of trypsin (Kaverin and Webster) or high trypsin concentrations (US 5,753,489), the process according to the present invention applies only half or less of the trypsin concentrations reported in the prior art. Moreover, a single addition of as little as 0.5 - 10 µg, preferably 2 - 5 µg trypsin per ml to the cell culture medium prior to or at the beginning of incubation of the infected host cells is sufficient to reach optimal infective virus titers. Inactivation experiments revealed that porcine or human recombinant trypsins are far less susceptible to inactivation by Vero or MDCK cells than bovine trypsin. Since bovine trypsin is most commonly used in the art it is rather likely that prior art literature unless explicitly mentioning another trypsin source, implicitly refers to bovine trypsin only. This would also help to explain the modes and concentrations of trypsin application recited, for instance, in Kaverin et al. and in US 5,753,489.

Using porcine or human rec trypsin or trypsinogen for initially supplementing the serum-free medium for Vero cell cultures according to the present invention therefore allows to use extremely low trypsin or trypsinogen concentrations and

thus prevents the need of labor-intensive and costly purification steps after harvesting of the virus-containing supernatant.

Another step that contributes to make the present process simple and therefore attractive to vaccine manufacturers is the addition of a single dose of highly active endonuclease to the cell culture medium prior to or at the beginning of incubation of the infected Vero cells for virus propagation. This endonuclease, preferably Benzonase™, is added once to the medium at a very low initial concentration of 2 - 30, preferably 5 - 15, Units per ml of medium and effectively clears the cell culture medium from free DNA and RNA originating mainly from the lysing or lysed host cells. The residual Benzonase enzyme concentration in the ready-for-use vaccine preparations obtained from the centrifuged supernatant remains at 5 ng or less per dose.

Benzonase™ is a trademark of Nycomed Pharma A/S Denmark and relates to an extracellular unspecific endonuclease obtained from *Serratia marcescens*. Benzonase is a genetically engineered endonuclease which degrades both DNA and RNA strands in many forms to small oligonucleotides. It promotes quick reduction of the viscosity of cell lysates, which facilitates ultracentrifugation. It reduces proteolysis and increases the yield in targeted protein and offers complete elimination of nucleic acids from, e.g. recombinant, proteins. It has an exceptionally high activity of 400,000 U/mg.

A third and important advantage of the present process is the factor time hence process costs. Due to the use of serum-free medium that does not contain proteins of animal origin and preferably no antibiotics, expensive and time-consuming purification procedures can be reduced to a minimum or even totally avoided. Also, because the addition of exogenous enzymes such as the protease (e.g. trypsin or trypsinogen) and nuclease (e.g. Benzonase) occurs once at the beginning of the virus propagation phase this saves plenty of time that the state-of-the-art methods require for post-incubation treatment of the virus-containing culture supernatant (e.g., HA activation, RNA/DNA digestion, protein purification, etc.).
Surprisingly, it turned out that the early addition of either or both of protease (e.g. trypsin or trypsinogen) and nuclease (e.g.Benzonase) to the virus-infected Vero-cell culture had no negative implications on the virus yield, which is

probably due to the very low enzyme concentrations applicable in the process of the present invention.

The present process of virus propagation is useful for the multiplication of various kinds of viruses, particularly influenza A viruses of the H3N2 subtype, but is also suitable for the isolation and reproduction of any epidemic or laboratory influenza virus strain, regardless of the kind of virus inoculum (e.g., blood serum sample, nasal wash, nasal swab, pharyngeal swab, saliva, etc.). Using the principles of this process, a number of influenza A and B vaccines has been produced which are part of the present invention and which are characterized in more detail in the subsequent Examples.
Also, protective efficacy as well as vaccine safety have been confirmed for the vaccines made according to the present invention, as will be demonstrated in the Examples.

The term "protein-free" or "free of non-serum proteins" as used herein in connection with the method of virus multiplication or propagation according to the present invention shall mean free of any functionally active protein. It shall not exclude, however, non-functional peptides as may originate from protein hydrolysates such as yeast extract or soya extract. Unless stated otherwise, the term "protein-free" shall neither exclude the presence of a protease and a nuclease enzyme at the concentrations disclosed and claimed herein.

In a preferred embodiment, the present invention relates to a simple, reliable and highly economic method for the manufacture of a whole-virus vaccine, preferably of an attenuated live vaccine, comprising the steps of:

a) infecting African Green Monkey Kidney (Vero) cells with a desired virus, wherein the Vero cells have been grown in and separated from a serum-free medium that is also free of non-serum proteins;

b) combining the infected cells with a suitable serum-free cell culture medium that is also free of non-serum proteins except for a protease and a nuclease; and

c) incubating the cells in the presence of said protease and said nuclease to allow for production of infectious virus and, simultaneously, for digestion of nucleic acid material released to the cell culture medium;

d) harvesting infectious virus by collecting virus-containing supernatant obtained from centrifugation of the cell culture; and

e) preparing a vaccine thereof comprising subjecting the virus-containing supernatant to at least one processing step selected from the group consisting of filtering, concentrating, freezing, freeze-drying, and stabilizing by addition of a stabilizing agent.

It is preferred that the virus used for propagation has never had any contact to a host substrate other than a Vero cell line. This will ensure best results with regard to immunogenic and antigenic identity of the initial virus (e.g. nasal swab isolate) and the viral progeny obtained after propagation.

It is also preferred that the virus used for propagation, particularly for the manufacture of a whole-virus vaccine, preferably an influenza attenuated live vaccine, is an influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, B/Vienna/99/ca37 and any attenuated variants and reassortants derived from any one of these strains. The genetic characteristics of the preferred virus strains, e.g. master strains, are disclosed in full detail in the subsequent Examples.

In another embodiment, the present invention refers to a whole-virus vaccine itself, preferably to an attenuated live vaccine, which in its ready-for-use form comprises essentially unmodified, optionally filtered and/or concentrated, virus-containing supernatant of a serum-free and protein-free Vero cell culture used for production of said virus. It is particularly preferred that the vaccine is produced according to the method of the present invention as disclosed and claimed herein.
This "one-step" vaccine, which does not require further processing, e.g., purification steps other than centrifugation and/or conventional filtration (i.e. not gel filtration), is compliant with the requirements for FDA approval.

The term "essentially unmodified" as used herein with regard to virus-containing supernatant in vaccine preparations according to the present invention shall refer to the composition of the supernatant as is at the time of harvesting the propagated virus, i.e. to the composition of the soluble components and ingredients present in the liquid phase of the supernatant. Minor alterations of the composition of ingredients as may occur due to steps of, for example, filtration, sterile filtration, centrifugation, concentration, drying, or freeze-drying

of the virus-containing supernatant, shall be regarded as falling within the scope of "essentially unmodified". Also, the term shall not exclude the presence of preserving and/or stabilizing agents usually applied in the art to vaccine preparations.

The whole-virus vaccines of the present invention may be used for the prophylactic or therapeutic treatment of viral infections, particularly of influenza virus infections. They may be administered as known in the art, e.g. intravenously, subcutaneously, intramuscularly or, most preferably, intranasally. The virus strains disclosed herein and the vaccines made thereof may, however, also be used as vectors or shuttles to present heterologous antigens to the immune system, e.g. antigens of viral envelope proteins such HIV-1 or hepatitis antigens.

Further preferred embodiments are defined in the dependent claims.

In order that the invention described herein may be more fully understood, the following Examples are set forth. They are for illustrative purposes only and are not to be construed as limiting this invention in any respect.

Example 1: Virus production

Cultivation of Vero /SF (=serum-free) cells:

SF-Medium: DMEM (Biochrom F0435), Ham's F12 (Biochrom F0815), 5mM L-Gln, 0.1% SF-supplement (a) or (b); antibiotics (only for first passage of virus isolation).

SF-Supplement: protein hydrolysate of non-animal origin, without functional proteins such as insulin, transferrin or growth factors:

a) 62.5 g hy-soy/UF, Quest 5X59100, to 500 g HQ-water, filtered with PES 0.2 µm filter;

b) 12.5 g hy-pep 1510, Quest, to 100 g HQ-water, filtered with PES 0.2 µm filter.

The content of a deep frozen (liquid nitrogen) disinfected (70% ethanol) ampule of WCB Vero cells was thawed and added to 9 ml of cold serum-free (SF) medium in a 10 ml tube and centrifuged for 10 min at 1000rpm (170 g). The

pellet was resuspended in SF-medium to a total of 30 ml, transferred to a 80 cm^2 Roux bottle and incubated at 37°C and 7%CO_2 for at least 15 min. Thereafter, the medium was removed and the cells were washed with approx. 0.1 ml/cm^2 PBS def.(= PBS without Ca^{2+} and Mg^{2+}). Addition of trypsin/EDTA-solution (8-10 µl/cm^2; 0.1% trypsin / 0.02% EDTA-solution) and incubation at room temperature for about 3 min. Detaching by gently pushing the Roux bottle against palm of the hand, addition of SF-medium and trypsin inhibitor (Sigma, T6522) at a quantity of about 1/5 of volume of the trypsin/EDTA solution. Repartition of the cell suspension to Roux bottles or roller bottles, incubation at 37°C and 9% CO_2.

MDCK cells were grown in DMEM/Ham's F12 + 2% FCS (heat inactivated); embryonated hen eggs were 11-12 days old and of SPF (specific pathogen free) origin.

Propagation of virus strains:

Old medium from roller bottles containing Vero cells was removed and cells were infected with virus by addition of 5 ml virus suspension in SF-medium to each roller bottle, resulting in an MOI (multiplicity of infection) of approximately 0.01. After incubation for 45 minutes at 33°C the virus inoculum was removed with a pipette. 90ml of SF-medium supplemented with 0.5 - 10, preferably 2 - 5 and most preferably 2 µg/ml porcine trypsin (supplier: AvP) or human recombinant trypsin or trypsinogen (own production) and 0.5 g/l sodium bicarbonate were added to each roller bottle and the bottles incubated at 33°C and 5% CO_2. For the production of attenuated live vaccine samples for use in animal testing and in human clinical trials the SF-medium was supplemented with trypsin and, additionally, with Benzonase™ at a concentration of 2 - 30, preferably 5 - 15, and most preferably 10 Units of Benzonase™ per ml of medium. Virus was harvested after 64 hours post infection by centrifugation of the culture supernatant for 5 min at 4000 rpm (3000g) at 10°C in 50 ml-tubes. The supernatant was pooled for each virus strain and stored at +4°C. Aliquots thereof were used for vaccine testing.

For storage purposes the virus preparations may be freeze-dried and stabilizer such as, for example, trehalose and lactalbumin enzymatic hydrolysate in HEPES buffer may be added. Reconstitution may be done with sterile water.

Example 2: Comparison of trypsin inactivation in cell cultures

Table 1: Trypsin inactivation in Vero vs. MDCK cell culture

	Vero / MDCK			
	0 h	**24 h**	**48 h**	**72 h**
bovine trypsin	0.314/0.314	0.199/0.239	0.110/0.201	0.026/0.203
porcine trypsin (high)	0.230/0.230	0.201/0.206	0.171/0.209	0.133/0.201
porcine trypsin (low)	0.129/0.129	0.108/0.118	0.081/0.099	0.054/0.116
human rec trypsin	0.097/0.097	0.054/0.088	0.026/0.080	0.008/0.076

Supernatants obtained from uninfected Vero cell cultures (grown in SF medium as described in Example 1) and MDCK cell cultures (grown in FCS-supplemented medium as described in Example 1) were tested for their capacity to inactivate trypsin of different origin that has been added to the supernatant at time = 0 h at equal concentrations each. Porcine trypsin has been applied in two different qualities (obtained from different manufacturers), i.e. with high or low activity. The results are presented in Table 1 and in Figures 1 and 2.

The data unambiguously show that bovine trypsin is rapdily inactivated in Vero cell culture supernatant and less rapidly in MDCK cell culture supernatant. Porcine and human rec trypsin (manufactured in our laboratories) remain fully active in MDCK supernatants while they are gradually inactivated in Vero supernatants at approximately half or less of the velocity of bovine trypsin inactivation. The difference of the porcine trypsins tested is only in the starting OD-level at 247 nm, while the inactivation characteristics are essentially identical for both lots of porcine trypsin.

Example 3: Comparison of various viral properties after growth on different host cell substrates

Virus propagation was carried out as described in Example 1 for the different host cell substrates. Each of the seven isolates recovered on Vero cells was reactive with human erythrocytes but not with chicken erythrocytes and none of them accumulated in embryonated eggs. On the other hand, all isolates recovered on MDCK cells were reactive both with chicken and human erythrocytes and were capable of growing in eggs. Although these differences were not seen in influenza A viruses of the H1N1 substype nor in influenza B

isolates (see subsequent Tables 3 and 4), it may nevertheless be assumed that cultivation of influenza viruses on Vero cells will maintain antigenic properties more properly than cultivation on other substrates.

Table 2: Characteristics of H3N2 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs
			chicken erys	human erys	
A/47/96	A/Johannesburg/ 33/94	Vero	-	+	-
		MDCK	+	+	+
A/7729/98	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1143/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1144/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1179/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1180/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1182/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+

From the data in Table 3 it appears that H1N1 influenza viruses may be less susceptible to adaptive selection, as the Vero and MDCK-grown isolates do not exhibit significant differences in their hemagglutination characteristics nor in their HA sequences. A similar conclusion may be drawn for the B isolates listed in Table 4.

The clinical starting material (e.g. serum samples, swabs) for virus isolation and replication was primarily obtained from:

1. Institute of Virology, Vienna, Austria (Prof. F. Heinz) 1995/96, 1996/97
2. Unité de Génétique Moléculaire des Virus Respiratoires, Institute Pasteur, Paris, France (Prof. S. van der Werf) 1996/97
3. Public Health Laboratory Service, London, UK (Dr. M. Zambon) 1996/97
4. Laboratoire Central de Virologie, Hôpitaux Universitaires de Genève, Geneva, Switzerland (Dr. W. Wunderli) 1996/97, 1997/98

5. Virus Unit, Queen Mary Hospital, Hong Kong (Dr. W.L. Lim) 1997/98

Table 3: Characteristics of H1N1 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		225
A/5389/95	A/Bayern/7/95	Vero	+	+	+	D
		MDCK	+	+	+	D
A/1035/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	G
		Swab				D
A/1131/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Swab				D
A/1134/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	n.t.
		Swab				D

Tabelle 4: Characteristics of B viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		198
B/4291/97	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/1/99	B/Beijing/184/93	Vero	+	+	+	T(g.s)
		MDCK	+	+	+	T(g.s)
		EGG	+	+	+	A
		Swab				T(g.s)

B/110/99	n.t.	Vero MDCK	+ +	+ +	+ +	identical
B/147/99	n.t.	Vero MDCK	+ +	+ +	+ +	identical
B/156/99	B/Beijing/184/93	Vero MDCK	+ +	+ +	+ +	identical
B/157/99	B/Beijing/184/93	Vero MDCK	+ +	+ +	+ +	identical

Table 5: Amino acid changes in HA, NA and M proteins of H3N2 influenza viruses isolated on different host systems

Isolate number	Changes at positions								
	HA						NA		M
	128	129	229	133	218	220	136	151	
A/47/96 Vero A/47/96 MDCK	T(g.s) A								
A/7729/98 Vero A/7729/98 MDCK		E G	R K						
A/1143/99 Swab A/1143/99 Vero A/1143/99 MDCK				N(g.s) N(g.s) D	G G E		n.t	n.t D G	n.t identical
A/1144/99 Swab A/1144/99 Vero A/1144/99 MDCK						R R G	n.t identical		n.t identical
A/1179/99 Swab A/1179/99 Vero A/1179/99 MDCK	identical						n.t identical		n.t identical
A/1180/99 Swab A/1180/99 Vero A/1180/99 MDCK	identical						n.t Q R	n.t	n.t identical
A/1182/99 Swab A/1182/99 Vero A/1182/99 MDCK	identical						n.t n.t n.t		n.t n.t n.t

The results show that with some isolates there was no alteration of the HA sequence of Vero or MDCK propagated viruses over the HA sequence directly obtained from the swab material by PCR amplification. In some other isolates

grown on MDCK cells the HA and/or NA sequences were deviating from the corresponding sequences obtained on Vero cells. The Vero-derived viruses did not show, however, any deviations in the HA sequence over the HA sequence of the swab isolates, where determined.

Table 6: Immunogenicity of Vero-, MDCK- and Egg-derived viruses for macaques

Animal number	Virus for immunization	Dose, PFU/ml	Serum HI titers
96	A/Vienna/47/96 V	$5x10^4$	256
88	A/Vienna/47/96 V	$5x10^4$	128
15	A/Vienna/47/96 V	$1.0x10^6$	128
95	A/Vienna/47/96 V	$1.0x10^6$	256
93	A/Vienna/47/96 M	$1.0x10^6$	16
128	A/Johannesburg/33/94 E	$5x10^6$	32
110	A/Vienna/157/97 V	$5x10^4$	128
78	A/Wuhan/359/95 E	$5x10^6$	32

The Macaques were immunized i.n. in the absence of anesthesia with 1 ml of virus suspension

V – Vero- isolated virus

M - MDCK -isolated viruses

E - egg isolated viruses

Table 7: Virulence of Vero- and MDCK- derived variants of A/Vienna/47/96 wt virus for ferrets

Viruses	Virus dose,	Number of animals with fever on day		
	PFU/ml	1	2	3
A/Vienna/47/96 Vero	$2x10^2$	FF	FFF	
	$1x10^3$	FFF	FFF	
A/Vienna/47/96 MDCK	$5x10^2$			
	$5x10^3$		FF	
	$5x10^4$	FF	F	F

Animals were immunized i.n. under ether narcosis with 1 ml of virus suspension.

N- normal temperature from 38.1°C to 39.9°C;

F- fever, more than 40.0°C.

The most surprising, yet important result in Table 6 is the very low immunogenicity of MDCK-derived A/Vienna/47/96 virus compared with the corresponding Vero-derived virus. It is no particular surprise that the egg-derived viruses show only poor immunogenicity.

Similarly, the results listed in Table 7 indicate that Vero-derived viruses are less, if at all, altered by adaptive selection on their host substrate in comparison to MDCK-derived viruses. This means that relative to the MDCK-derived viruses the Vero-derived viruses maintain more or even all of the immunologically relevant, particularly antigenic, properties of the original virus.

Example 4: Vaccine production with preferred strains

The process described in Example 1 was also used for the production of vaccine samples for animal testing and human clinical studies. It is understood that the process of virus propagation described therein also encompasses variations that could be suggested or applied by a person of ordinary skills in the art without inventive input and as long as the variations do not change the sense of the present invention as described herein and in the claims.

Vaccine samples containing one or more of the preferred influenza A or B wildtype strains, master strains or reassortant strains (that are subsequently described in more detail) were exclusively produced using the continuous Vero cell line as the host cell system (unless for purposes of comparison with samples obtained from other host substrates) in serum-free medium additionally supplemented with the nutritional ingredients and enzymes as described in Example 1.

Some methods suitable for modifying wildtype viruses including the methods of attenuation (e.g., temperature sensitivity), cold adaptation and reassortment are known in the art and extensively reviewed, for instance, in WO 99/64068.

Further characteristics of the two most preferred influenza A and B master strain candidates useful for attenuated live vaccine production, e.g., by 6/2 reassortment with the HA and NA genes of actual epidemic influenza viruses recommended by the WHO, are given in the following Tables 8 - 13.

Table 8: Characteristics of master strain candidates for live influenza vaccines

	Influenza A *A/Singapore/1/57/ca* H2N2	Influenza B *B/Vienna/1/99/ca*
Passage history	A/Singapore/1/57 wt egg derived H2N2 20 passages at 37°C on Vero/SF cells 25 passages at 25°C on Vero/SF cells	B/Vienna/1/99 wt Vero derived 1 additional passage at 33°C on Vero/SF cells 22 passages at 25°C on Vero/SF cells
Method of attenuation	Serial passages at optimal and suboptimal temperature on heterologous system	Serial passages at optimal and suboptimal temperature on heterologous system
Phenotypic markers	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs
Genotypic markers	Mutations: 13 (8 coding) PB2 3 (2 coding) PB1 2 (1 coding) PA 4 (3 coding) NP 1 M 2 (2 coding) NS 1	Mutations: 5 (3 coding) PB2 0 PB1 1 PA 0 NP 2 (1 coding) M 1 NS 1

Table 9: Full Sequence of the 8 genome segments and of the 10 corresponding proteins of strain A/Singapore/1/57/ca

A/Singapore/1/57/ca (H2N2)			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 1	PB2	SEQ ID No. 9
2	SEQ ID No. 2	PB1	SEQ ID No. 10
3	SEQ ID No. 3	PA	SEQ ID No. 11
4	SEQ ID No. 4	HA	SEQ ID No. 12
5	SEQ ID No. 5	NP	SEQ ID No. 13
6	SEQ ID No. 6	NA	SEQ ID No. 14
7	SEQ ID No. 7	M1	SEQ ID No. 15
		M2	SEQ ID No. 16
8	SEQ ID No. 8	NS1	SEQ ID No. 17
		NS2	SEQ ID No. 18

ca - cold adapted

It shall be noted, however, that the genome segments No. 4 and 6, i.e., the HA and NA genes, are not required to characterize the influenza A master strain candidates, because these genes will be exchanged for the corresponding genes of actual epidemic influenza viruses (as mentioned hereinbefore). The features important for the safety of a vaccine, e.g. temperature sensitivity, or features that allow intranasal administration of a vaccine, namely cold adaptation (because the average temperature in a nose is lower than the usual body temperature), are primarily caused by mutations in the remaining 6 genome segments.

The following Table 10 lists the mutations in the genome segments of A/Singapore/1/57/ca compared to the corresponding wildtype strain A/Singapore/1/57/wt.

Table 10: Mutations in the genome segments of attenuated, temperature sensitive, cold adapted influenza strain A/Singapore/1/57/ca compared to A/Singapore/1/57/wt strain

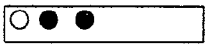
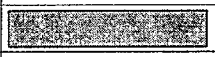
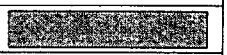
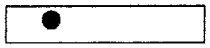
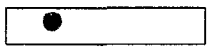
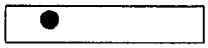
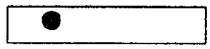
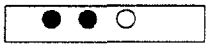
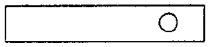
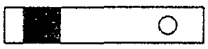
RNA	Length	Nucleotides changed			Protein	Length	Amino acids changed		
segment	(n'ds)	position	wt	ca		(aa)	position	wt	ca
1	2341	252	a	g	PB2	771	-	-	-
		581*	t	c			185	I	T
		1046*	g	t			340	R	I
2	2341	1279*	t	a	PB1	757	419	L	I
		1965	a	c			-	-	-
3	2233	707*	a	t	PA	716	228	I	N
		1425	t	a			-	-	-
		1537*	a	g			505	V	I
		1819*	g	c			598	Q	E
5	1565	210	g	a	NP	506	-	-	-
7	1027	327*	g	a	M1	252	101	R	K
		499*	g	c			158	Q	R
					M2	97	-	-	-
8	890	813	a	g	NS1	237	-	-	-
					NS2	121	-	-	-

Total number of mutations - 13 (8 coding)

* coding mutations

Preferred variants of A/Sing/1/57/ca comprise the ones listed in the following Table 11, wherein "Δ" means "del" or "delta" and stands for a mutant that contains at least one "deletion" in its NS gene segment.

Table 11: Preferred variants of A/Sing/1/57/ca

	A/Sing/1/57/ca	Sing ca/ ΔNS 87	Sing ca/ ΔNSPR8	Sing ca/ NS124PR8
PB2 (Sing ca*)				
PB1 (Sing ca*)				
PA (Sing ca*)				
HA				
NP (Sing ca*)				
NA				
M1,2 (Sing ca*)				
NS1,2 (Sing ca*)		 del 87 aa NS1		
NS1,2 (PR8**)			 del NS1	 Stop 124 NS1
Phenotypes				
ca	+	+	+	+
ts	+	+	+	+
IFN-induct.	–	+/–	+	+
IFN-sensit	–	+	+	–

* genome segment originating from A/Singapore/1/57/ca

** genome segment originating from influnza A/PR8/34

ca - cold adapted; ts - temperature sensitive;

aa - amino acid(s)

IFN-induct. - strain causes interferon release in host substrates that are able of IFN production, as well as in animal or human immune systems upon administration.

IFN-sensit. - strain is sensitive towards interferon; replication in IFN producing systems is reduced or stopped.

Sing ca/ΔNS 87 - strain A/Singapore/1/57/ca containing deletion of 87 amino acids in NS1 gene at aa position 36-123.

Sing ca/ΔNSPR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 (herein also abbreviated "PR8") which contains a deletion of the entire NS1 gene.

Sing ca/NS124PR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 which contains a stop codon at aa position 124 of the NS1 gene.

+/- means that the phenotype needs further clarification and can not yet be unambiguously defined.

The following Tables 12, 13 and 13A refer to preferred influenza B master strain candidates and to variations and reassortants, respectively, thereof.

Table 12: Full Sequence of the 8 genome segments and of the 11 corresponding proteins of strain B/Vienna/1/99/ca

B/Vienna/1/99/ca			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 19	PB2	SEQ ID No. 27
2	SEQ ID No. 20	PB1	SEQ ID No. 28
3	SEQ ID No. 21	PA	SEQ ID No. 29
4	SEQ ID No. 22	HA_0	SEQ ID No. 30
5	SEQ ID No. 23	NP	SEQ ID No. 31
6	SEQ ID No. 24	NB	SEQ ID No. 32
		NA	SEQ ID No. 33
7	SEQ ID No. 25	M1	SEQ ID No. 34
		BM2	SEQ ID No. 35
8	SEQ ID No. 26	NS1	SEQ ID No. 36
		NS2	SEQ ID No. 37

ca - cold adapted

The original strain B/Vienna/1/99 was isolated on Vero cell culture grown with serum-free medium in February 1999 in Vienna, Austria from a 12 year old female with acute influenza. It was rated as B/Beijing/184/93-like by the Center for Disease Control (CDC), Atlanta, USA. After an additional passage at 33°C the wildtype strain – designated as B/Vienna/1/99 wt – was attenuated by 22

serial passages at 25°C using the same cell culture system. The plaque purification was done at 25°C for the first and at 33°C for the following four rounds. The derived plaque purified clone was amplified and stored at -70°C, designated as B/Vienna/1/99 ca or briefly BV22. The identity as a B/Beijing/184/93-like virus was confirmed by HI-assay with standard anti-serum from NIBSC.

Table 13: Mutations in B/Vienna/1/99/ca (=BV22) compared to B/Vienna/1/99/wt (BVie) 1. passage on Vero/SF

Segment (lenght in nucleotides)	Nucleotides changed			Protein (length in amino acids)	Amino acids changed		
	Posi-tion	BVie	BV22		Posi-tion	BVie	BV22
1 (2396)	-	-	-	PB2 (770)	-	-	-
2 (2369)	594	T	C	PB1 (752)	-	-	-
3 (2305)	-	-	-	PA (726)	-	-	-
4 (1882)	457 1299 1595	G G G	A T A	HA_0 (584)	142 422 521	A K G	T N E
5 (1844)	128 330	C T	T C	NP (560)	23 -	S -	F -
6 (1557)	- 823 1135	- G T	- A C	NB (100) NA (466)	- 257 361	- R I	- Q T
7 (1190)	- 831	- A	- G	M1 (248) BM2 (109)	- 21	- M	- V
8 (1097)	116 -	G -	A -	NS1 (281) NS2 (122)	25 -	A -	T -

Table 26: Characterization of B/Vienna/1/99 wt according to Los Alamos National Library influenza database (db) (Web-adress: www.flu.lanl.gov)

B/Vienna/1/99 wt gene coding for	Accession Nr. amino acid seq.	Accession Nr. nucleotide seq	Remarks
PB2, segment 1	ISDACH017	ISDNCHB017	in db listed as segment 2
PB1, segment 2	ISDACH016	ISDNCHB016	in db listed as segment 1
PA, segment 3	ISDACH015	ISDNCHB015	
HA, segment 4	ISDACH018	ISDNCHB018	
NP, segment 5	ISDACH013	ISDNCHB013	
NA, segment 6	ISDACH012	ISDNCHB012	
M, segment 7	ISDACH011	ISDNCHB011	
NS, segment 8	ISDACH014	ISDNCHB014	

In addition, further passaging of strain B/Vienna/1/99 ca for 15 additional passages (i.e. a total of 37 passages on serum-free Vero cell culture) resulted in a mutant B/Vienna/1/99 ca37 (abbreviated BV37) with properties even superior to the ones of BV22. This mutant contains an increased number of mutations vis-à-vis BV22 and appears to be the currently most promising candidate for the production of a whole-virus vaccine, particularly for an attenuated influenza live vaccine, based on a non-recombinant influenza virus mutant. The additional mutations are listed in Table 13A below:

Table 13 A: Mutations for BV22 and BV37 compared to B/Vienna/1/99 wt 1st passage on Vero/SF

Segment (lenght in nucleotides)	Nucleotides changed				Protein (length in amino acids)	Amino acids changed			
	Pos.	BVie	BV22	BV37		Pos.	BVie	BV22	BV37
1 (2396)	-	-	-	-	PB2 (770)	-	-	-	-
2 (2369)	594	T	**C**	**C**	PB1 (752)	-	-	-	-
(BV37: 2370)	2348	-	-	**A**		-	-	-	-
3 (2305)	-	-	-	-	PA (726)	-	-	-	-
4 (1882)	457	G	A*	A*	HA_0 (584)	142	A	T+	T+
	1122	C	C	**A**		363	F	F	**L**
	1299	G	**T**	G		422	K	**N**	K
	1595	G	**A**	**A**		521	G	**E**	**E**
5 (1844)	128	C	**T**	**T**	NP (560)	23	S	**F**	**F**
	212	C	C	T		51	P	P	**L**
	330	T	C#	C#		-	-	-	-
6 (1557)	-	-	-	-	NB (100)	-	-	-	-
	823	G	**A**	G	NA (466)	257	R	**Q**	R
	1135	T	C●	C●		361	I	T●	T●
7 (1190)	24	G	G	**A**	M1 (248)	-	-	-	-
	831	A	**G**	**G**		-	-	-	-
	831	A	**G**	**G**	BM2	21	M	**V**	**V**
	1029	A	A	**G**	(109)	87	I	I	**V**
8 (1097)	116	G	**A**	**A**	NS1 (281)	25	A	**T**	**T**
	-	-	-	-	NS2 (122)	-	-	-	-

Comparison with influenza sequence database 13.2. 2001 (www.flu-lanl.gov):

a) unique mutations underlined in bold type;

b) mutations common with:

* B/Lee/40, B/Osaka/70, B/Kadoma/1076/99 (resulting amino acid: I)

\+ B/Lee/40, B/Osaka/70

often: B/Lee/40, B/Ann Arbor/1/66 ca & wt, B/Singapore/222/79, B/North Dakota/83, B/Norway/1/84, B/Ibaraki/2/85, B/Ann Arbor/1/86, B/Victoria/2/87, B/Aichi/5/88

- B/Kanagawa/73

It shall be understood that the influenza A and B master strains according to the present invention shall not be limited to the features and genetic characteristics explicitly listed in the tables herein but shall also comprise minor variations thereof as long as such variations are in the sense of the present invention and do not subtantially alter any one of the functional features of the virus. Such variations may occur, for instance, due to additional steps of virus multiplication or propagation (e.g. for the purpose of obtaining material for sequence analyses).

Moreover, the gene sequences listed herein include the primer sequences (located at the beginning and at the end of each genome segment) that were used along with the present invention, which primer sequences may differ from the corresponding true sequences of the viral genome segments of either or both the wildtype and the attenuated virus strains.

Example 5: Vaccine safety and efficacy

The subsequent data confirm temperature sensitivity and vaccine safety for influenza vaccines manufactured according to the present invention, e.g., as described in Example 1.

Table 14: Antibody response of mice after one intranasal immunisation without narcosis

Viruses	Number of responders[1]	GMT[3]	Protection after challenge[2]
PR8/Sing ca -2/6	0/6	< 4	5/6
PR8/Sing ca -ΔNS	4/6	6.7	5/6
PR8-wt	5/6	16.0	5/6

1 - number of animals with positive HI titer > 1:4

2 - number of animals without detectable virus in the lungs

3- Geometric mean titer of antibodies in serum

PR8wt – influenza strain A/PR/8/34 wildtype (H1N1), pathogenic for mice

PR8/Sing ca-2/6 - is the reassortant between attenuated influenza strain A/Sing/1/57 ca and PR8 wt, containing 2 genes (HA and NA) from PR8wt virus and all other genes from A/Sing/1/57 ca.

PR8/Sing-ΔNS contains HA and NA genes from PR8wt, five genes from A/Sing/1/57 ca and the NS gene of PR8 origin lacking the NS1 coding sequence (NS1 deletion or knockout).

Table 15: Antibody response and protection of mice after intranasal immunisation with different variants of A/Singapore/1/57 virus (under narcosis)

Viruses	Responders[1]		GMT after two immunisations	Protection after challenge[4]
	1-st immunisation	2-nd immunisation		
A/Sing/1/57/wt va[2]	9/9	9/9	103.9	9/9
A/Sing/1/57/ca[3]	8/10	10/10	55.7	8/10
A/Sing /57/ΔNS 87	1/10	10/10	27.9	8/10

[1] - number of animals with positive HI titer > 1:4

[2] - va- Vero-adapted

[3] - ca - cold-adapted

[4] - number of animals without detectable virus in the lungs

Table 16: Reproduction of wt, va and ca variants of A/Singapore/1/57 in mouse lungs[a]

Viruses	Virus titer in mouse lungs post infection on day, PFU/ml[b]		
	2	4	6
A/Singapore/1/57/wt	1.6×10^6	2.2×10^5	1.4×10^3
A/Singapore/1/57/wt va	2.5×10^6	2.1×10^6	1.0×10^2
A/Singapore/1/57/ca	< 10	< 10	< 10

[a] Mice were infected i.n. with 50 μl of virus fluid with a titer 1.0×10^6 PFU/ml.

[b] PFU/ml of 10% tissue suspension, titrated on MDCK cells.

Table 17: Virulence of wt and ca variants of A/Singapore/1/57 virus for ferrets

Viruses	Number of animals with fever post infection on day		
	1	2	3
A/Singapore/1/57 wt	FFF	NNN	NNN
A/Singapore/1/57 ca	NNN	NNN	NNN

Rectal temperature of animals was recorded twice a day and characterized as follows:

N - normal temperature from 38.1°C to 39.9 °C

F - fever, more than 40.0°C.

Each group consisted of 3 animals, which were immunized i.n. under ether narcosis with 1 ml of virus fluid with a titer of $2x10^6$ PFU/ml.

Table 18: Reproduction of 2/6 reassortant of A/Hong Kong/1035/98 wt and A/Singapore/1/57/ca in mouse lungs[a]

Viruses	Virus titer in mouse lungs on day 2-6 post infection, PFU/ml[b]		
	2	4	6
A/Hong Kong/1035/98 wt H1N1	$6.8x10^4$	$2.0x10^4$	< 10
A/Singapore/1/57/ca x A/Hong Kong/1035/98 wt	< 10	< 10	< 10

[a] Mice were infected i.n.under ether narcosis with 50 µl of virus fluid.

[b] PFU/ml of 10% tissue suspension, titrated on Vero/SF cells, data are given as mean value for 6 mice (the lungs of each animal were treated separately).

The reassortant contains the HA and NA genes from A/Hong Kong/1035/98 wt wildtype and the other 6 genes from A/Singapore/1/57/ca.

Table 19: Virulence of 6/2 reassortant of A/Vienna/47/96 wt and A/Singapore/1/57/ ca for ferrets

Viruses	Virus subtype	Number of animals with fever on day			
		1	2	3	Rhinitis[b]
Master strain A/Singapore/1/57/ ca	H2N2	NNN	NNN	NNN	±
Epidemic virus A/Vienna/47/96 wt	H3N2	NNN	FFF	FFF	+ + +
Reassortant A/Singapore/1/57/ca x Vienna/47/ 96 wt	H3N2	NNN	NNN	NNN	±

Animals were immunized i.n. under ether narcosis with 1 ml of virus, $2x10^6$ PFU/ml.

N- normal temperature from 38.1°C to 39.9°C;

F- fever, more than 40.0°C.

[b] + + + - severe rhinitis

± absence of rhinitis

The results presented in Tables 16 to 19 clearly demonstrate the safety of the vaccines containing the attenuated, temperature sensitive master strain or, in case of reassortants, of the vaccines based on the reassorted viruses composed of the "backbone" of the attenuated, temperature sensitive master strain (6 genes) and the HA and NA genes from, e.g., the pathogenic wildtype strain A/Hong Kong/1035/98 wt.

Table 20: Ts and ca phenotype of B/Vienna/1/99

Virus	PFU/ml on Vero cells at	PFU/ml on MDCK cells at	
	25°C	33°C	39°C
B/Vienna/1/99 wt	< 300	$4x10^6$	$4x10^5$
B/Vienna/1/99 ca (BV22)	$1x10^6$	$2.4x10^6$	< 20

Table 21: Genetic stability of the ts phenotype of B/Vienna/1/99 ca

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	$4x10^6$	$4x10^5$
B/Vienna/1/99 ca (BV22)	$2.4x10^6$	< 20
B/Vienna/1/99 ca (BV22) after 5 passages at 33°C	$8x10^5$	< 20

The strain BV22 was passaged five times at high MOI on Vero cells. Then the ts-phenotype was controlled again. The strain remained tmperature senssitive as can be seen in Table 21.

Table 22: Virulence of B/Vienna/1/99 ca and wt in mouse lungs

		PFU/ml* at day post infection		
Virus	organ	2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	$1x10^2$	$1x10^2$	20
B/Vienna/1/99 wt	lung	$8x10^4$	$7x10^3$	$4.4x10^3$
	nose	$3.8x10^4$	$3.4x10^4$	$1.4x10^4$

* 9 OF1 mice per strain were immunized intranasally under ether narcosis with 10^5 PFU. At the indicated days post infection 3 mice per group were sacrificied. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

The data show that moderate reproduction of the ca master strain candidate BV22 was possible in the nasal mucosa while the ts property of the virus prevented reproduction in the lungs.

Table 23: Ts and ca phenotype of the reassortant influenza B strain

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	$4x10^6$	$4x10^5$
B/USSR/69 wt	$1.6x10^6$	$4x10^4$
B/Vienna/1/99 ca (BV22)	$1.4x10^6$	< 20
BV22 x B/USSR/69 (6/2)	$8x10^6$	< 20

A 6/2 reassortant strain containing HA and NA of the wild type influenza strain B/USSR/69 wt and the other 6 genome segments from B/Vienna/1/99 ca (BV22) was established. The origin of the hemagglutinin was tested by HI-assay, all other genome segments by RT-PCT and restriction analysis using methods known in the art.

Table 24: Virulence of the reassortant influenza B strain in mouse lungs

		PFU/ml* at day post infection		
Virus	organ	2	3	4
B/Vienna/1/99 ca	lung	< 20	< 20	< 20
(BV22)	nose	< 20	$1x10^2$	40
B/USSR/69 wt	lung	$1.8x10^5$	$4x10^5$	$2.4x10^4$
	nose	$1.6x10^5$	$2x10^5$	$1.6x10^5$
BV22 x B/USSR/69 wt	lung	< 20	< 20	< 20
(6/2)	nose	$2.8x10^3$	$2x10^3$	$4x10^2$

* 9 OF1 mice per strain were immunized intranasally under ether narcosis with 10^5 PFU. At the indicated days post infection 3 mice per group were sacrificied. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

Example 6: Clinical study

The following vaccines (in the form of nasal sprays) were produced according to the present invention (e.g. as described in Example 1) for intranasal delivery. Composition per ml (after reconstitution of freeze-dried material):

(1) Placebo: 2x SF-medium, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

(2) Vero-Vac H1: A/Beijing/262/95 (H1N1)-like preparation comprising $4.3x10^7$ $TCID_{50}$ of 6/2 reassortant A/Singapore/1/57/ca with A/Hong Kong/1035/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

(3) Vero Vac H3: A/Sidney/5/97 (H3N2)-like preparation comprising $2.1x10^7$ $TCID_{50}$ of 6/2 reassortant A/Singapore/1/57/ca with A/SW/7729/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

(4) Russian trivalent vaccine (live influenza vaccine for adults):
A/17/Beijing/95/25 (H1N1) $1.1x10^8$ EID_{50}

A/17/Sidney/97/76 (H3N2) $2.3x10^7$ EID_{50}

B/60/Petersburg/95/20 $1.1x10^7$ EID_{50}

(5) Monovalent Vero vaccine BV22: B/Beijing/184/93 - like preparation comprising $2x10^6$ $TCID_{50}$ of master strain candidate B/Vienna/1/99/ca (=BV22); 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

The vaccines were administrated to 13 volunteers per each vaccination group. 550 µl of reconstituted vaccine (or placebo, respectively) were given intranasally to each patient on day 0 and for a second time on day 22 ± 1. The results are summarized in Table 25 below.

Safety results:

The total number of adverse events (AE) during five days after the first and second vaccination was 14 including 9 mild and 4 moderate AE. Only one volunteer showed severe AE, comprising an increase in body temperature up to 38.8°C within 3 hours after the first vaccination without any local or systemic symptoms. During the next four hours his temperature became normal again. After the first vaccination 7 AE were observed. One of them was local and six were systemic. After the second vaccination 2 local and 5 systemic AE were observed.

No significant difference in terms of safety was revealed between the groups of the study including the one with placebo. No serious AE related to the vaccination were observed except for the one mentioned above. Two of the moderate AE occurred in the H3N2 group (temperature elevation up to 37.6° and acute pharyngitis on day 3 in one volunteer; nasal obstruction, discomfort in the throat on day 22-24 and temperature elevation up to 37.5°C in another volunteer), and one in the H1N1 group (pain in the throat, rhinitis from day 22-26, temperature elevation up to 37 - 37.8°C between days 22-24).

Table 25: Response of seronegative volunteers to Vero Vac vaccines and to a trivalent Russian cold-adapted egg derived vaccine

No	Vaccine for immunization	Virus dose, $TCID_{50}$/ml or EID_{50}/ml	No. of volunteers	% of volunteers with at least 4-fold increase of serum HAI antibody titre to antigens		
				H1N1	H3N2	B
1	Placebo		13		(8)	
2	Vero Vac H1 (H1N1)	$4.3x10^7$	13	38		
3	Vero Vac H3 (H3N2)	$2.1x10^7$	13		67	
4	Russian trivalent vaccine: A/17/Beijing/95/25 H1N1 A/17/Sidney/97/76 H3N2 B/60/Petersburg/95/20	 $1.1x10^8$ $2.3x10^7$ $1.1x10^7$	13	 46	 8	 31
5	Vero vaccine BV22	$2x10^6$	13			33

(8) patient developed spontaneous infection during course of study.

The results obtained from the clinical study thus confirm a very good safety of the vaccines produced according to the present invention and using the preferred influenza A and B master strain candidates of the present invention.

CLAIMS

We claim

1. A method for the manufacture of a whole-virus vaccine, preferably an attenuated live vaccine, comprising the steps of:

a) infecting African Green Monkey Kidney (Vero) cells with a desired virus, wherein the Vero cells have been grown in and separated from a serum-free medium that is also free of non-serum proteins;

b) combining the infected cells with a suitable serum-free cell culture medium that is also free of non-serum proteins except for a protease and a nuclease; and

c) incubating the cells in the presence of said protease and said nuclease to allow for production of infectious virus and, simultaneously, for digestion of nucleic acid material released to the cell culture medium;

d) harvesting infectious virus by collecting virus-containing supernatant obtained from centrifugation of the cell culture; and

e) preparing a vaccine thereof comprising subjecting the virus-containing supernatant to at least one processing step selected from the group consisting of filtering, concentrating, freezing, freeze-drying, and stabilizing by addition of a stabilizing agent.

2. The method according to claim 1, which does not involve a step of protein separation or purification.

3. The method according to claim 1 or 2, which does not involve a step of chromatographic separation or purification, and preferably does not contain any purification step other than centriguation and/or filtration.

4. The method according to any one of claims 1 to 3, which comprises at least one step of sterile filtration of the virus-containing supernatant.

5. The method according to any one of claims 1 to 4, wherein the nuclease has DNAse and/or RNAse activity, and preferably is Benzonase.

6. The method according to any one of claims 1 to 5, wherein the protease and the nuclease are added to the cell culture medium once prior to or at the beginning of incubation of the infected cells.

7. The method according to any one of claims 1 to 6, wherein the protease comprises trypsin and/or trypsinogen of human recombinant or porcine origin which is present in the cell culture medium at an initial concentration of 0.5 - 10, preferably 2 - 5 µg per ml medium.

8. The method according to any one of claims 1 to 7, wherein the cell culture medium comprises nuclease at an initial concentration of 2 to 30, preferably 5 to 15, U per ml of medium.

9. The method according to any one of claims 1 to 8, wherein the incubation in step (a) is carried out for 10 to 120 minutes, preferably for 30 to 60 minutes.

10. The method according to any one of claims 1 to 9, wherein the virus is selected from the group consisting of a wildtype virus, a primary isolate directly obtained from an infected individual, a recombinant virus, an attenuated virus, a Vero adapted virus, a cold-adapted virus, a temperature-sensitive virus, and a reassortant virus.

11. The method according to any one of claims 1 to 10, wherein the virus is an influenza A virus, preferably of subtype H3N2 or H1N1, or an influenza B virus.

12. The method according to any one of claims 1 to 11, wherein the virus has an interferon inducing and/or interferon sensitive phenotype.

13. The method according to any one of claims 1 to 12, wherein the virus is an influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, B/Vienna/99/ca37 and any attenuated variants and reassortants derived from any one of these strains.

14. A whole-virus vaccine, preferably an attenuated live vaccine, characterized in that in its ready-for-use form it comprises essentially unmodified, optionally filtered and/or concentrated, virus-containing supernatant of a serum-free and protein-free Vero cell culture used for production of said virus.

15. The vaccine according to claim 14, characterized in that it selectively agglutinates human erythrocytes but not chicken erythrocytes.

16. The vaccine according to claim 14 or 15, characterized in that it contains a suitable stabilizing agent.

17. The vaccine according to any one of claims 14 to 16, characterized in that it is in the form of a liquid, freezed or freeze-dried preparation, optionally suitable for intranasal delivery.

18. The vaccine according to any one of claims 14 to 17, characterized in that it is a live attenuated vaccine, preferably comprising whole influenza virus.

19. The vaccine according to any one of claims 14 to 18, characterized in that it comprises at least one influenza virus having a phenotype with one or more characteristics selected from the group consisting of cold adapted, temperature sensitive, interferon inducing, interferon sensitive.

20. The vaccine according to claim 18, wherein the influenza virus is selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains.

21. The vaccine according to claim 14, obtainable by a method of manufacture as defined in any one of claims 1 to 13.

22. A whole-virus vaccine, preferably an attenuated live vaccine, comprising at least one influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains.

23. The vaccine according to claim 21, characterized in that it selectively agglutinates human erythrocytes but not chicken erythrocytes.

24. The vaccine according to claim 22 or 23, obtainable by a method of manufacture according to any one of claims 1 to 13.

25. Use of a vaccine defined in any one of claims 14 to 24 for prophylactic or therapeutic administration against viral infection.

26. Use of at least one influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains, for the manufacture of a vaccine, preferably for the manufacture of a live attenuated influenza vaccine.

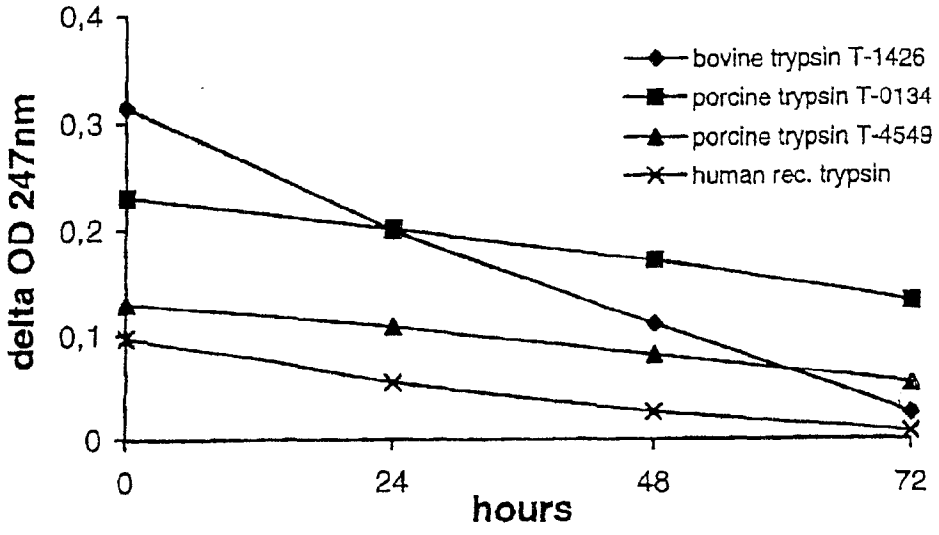

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Fig. 1

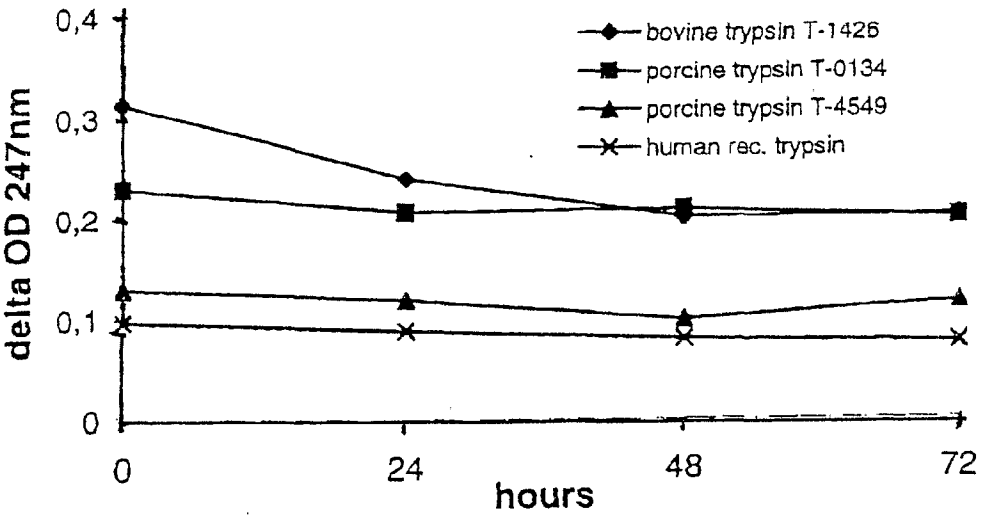
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Fig. 2

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cattctcctg ttcacagcag tgagagggga ccagatatgc attggatacc atgccaataa 120
ttccacagag aaggtcgaca caattctaga gcagaacgtc actgtgactc atgccaagga 180
cattcttgag aagacccata acggaaagtt atgcaaacta aacggaatcc ctccacttga 240
actaggggac tgtagcattg ccggatggct ccttggaaat ccagaatgtg ataggcttct 300
aagtgtgcca gaatggtcct atataatgga gaaagaaaac ccgagagacg gtttgtgtta 360
tccaggcagc ttcaatgatt atgaagaatt gaaacatctc ctcagcagcg tgaaacattt 420
cgagaaagta aagattctgc ccaaagatag atggacacag catacaacaa ctggaggttc 480
acgggcctgc gcggtgtctg gtaatccatc attcttcagg aacatggtct ggctgacaaa 540
gaaagaatca aattatccgg ttgccaaagg atcgtacaac aatacaagcg gagaacaaat 600
gctaataatt tgggggggtgc accatcccaa tgatgagaca gaacaaagaa cattgtacca 660
gaatgtggga acctatgttt ccgtaggcac atcaacattg aacaaaaggt caaccccaga 720
catagcaaca aggcctaaag tgaatggact aggaagtaga atggaattct cttggaccct 780
attggatatg tgggacacca taaattttga gagtactggt aatctaattg caccagagta 840
tggattcaaa atatcgaaaa gaggtaattc agggatcatg aaaacagaag gaacacttga 900
gaactgtgag accaaatgcc aaactccttt gggagcaata aatacaacat tgccttttca 960
caatgtccac ccactgacaa taggtgagtg ccccaaatat gtaaaatcgg agaagttggt 1020
cttagcaaca ggaccaagga atgttcccca gattgaatca agaggattgt ttggggcaat 1080
agctggtttt atagaaggag gatggcaagg aatggttgat ggttggtatg gataccatca 1140
cagcaatgac cagggatcag ggtatgcagc agacaaagaa tccactcaaa aggcatttga 1200
tggaatcacc aacaaggtaa attctgtgat tgaaaagatg aacacccaat ttgaagctgt 1260
tgggaaagaa ttcagtaact tagagagaag actggagaac ttgaacaaaa agatggaaga 1320
cgggtttcta gatgtgtgga catacaatgc tgagcttcta gttctgatgg aaaatgagag 1380
gacacttgac tttcatgatt ctaatgtcaa gaatctgtat gataaagtca gaatgcagct 1440
gagagacaac gtcaaagaac taggaaatgg atgttttgaa ttttatcaca aatgtgatga 1500
tgaatgcatg aatagtgtga aaaacgggac gtatgattat cccaagtatg aagaagagtc 1560
taaactaaat agaaatgaaa tcaaaggggt aaaattgagc agcatggggg tttatcaaat 1620

ccttgccatt tatgctacag tagcaggttc tctgtcactg gcaatcatga tggctgggat 1680
ctctttctgg atgtgctcca acgggtctct gcagtgcagg atctgcatat gattataagt 1740
cattttataa ttaaaaacac ccttgtttct act 1773

<210> 5
<211> 1565
<212> DNA
<213> Influenza virus A/Singapore/1/57/ca

<400> 5
agcaaaagca gggtagataa tcactcactg agtgacatca aaatcatggc gtcccaaggc 60
accaaacggt cttatgaaca gatggaaact gatggggaac gccagaatgc aactgaaatc 120
agagcatccg tcgggaagat gattgatgga attggacgat tctacatcca aatgtgcacc 180
gaacttaaac tcagtgatta tgaggggcga ctgatccaga acagcttaac aatagagaga 240
atggtgctct ctgcttttga cgagaggagg aataaatatc tggaagaaca tcccagcgcg 300
gggaaggatc ctaagaaaac tggaggaccc atatacaaga gagtaaatgg aaagtggatg 360
agggaactcg tcctttatga caaagaagaa ataaggcgaa tctggcgcca agctaataat 420
ggtgatgatg caacagctgg tctgactcac atgatgatct ggcattccaa tttgaatgat 480
acaacatacc agaggacaag agctcttgtt cgcaccggaa tggatcccag gatgtgctct 540
ttgatgcagg gttcgactct ccctaggagg tctggagccg caggcgctgc agtcaaagga 600
gttgggacaa tggtgatgga gttgatcagg atgatcaaac gtgggatcaa tgatcggaac 660
ttctggagag gtgagaatgg gcggaaaaca aggattgctt atgagagaat gtgcaacatt 720
ctcaaaggaa aatttcaaac agctgcacaa agagcaatga tggatcaagt gagagaaagc 780
cggaacccag gaaatgctga gatcgaagat ctcatctttc tggcacggtc tgcactcata 840
ttgagagggt cagttgctca caaatcttgt ctgcctgcct gtgtgtatgg aactgccgta 900
gccagtgggt acgacttcga aaaagaggga tactctttag tagggataga ccctttcaaa 960
ctgcttcaaa acagccaagt atacagccta atcagaccga acgagaatcc agcacacaag 1020
agtcagctgg tgtggatggc atgcaattct gctgcatttg aagatctaag agtatcaagc 1080
ttcatcagag ggaccaaagt aatcccaagg gggaaacttt ccactagagg agtacaaatt 1140
gcttcaaatg aaaacatgga tactatggaa tcaagtactc ttgaactgag aagcaggtac 1200
tgggccataa ggaccagaag tggaggaaac actaatcaac agagggcctc tgcaggtcaa 1260
atcagtgtac aacctacgtt ttctgtgcaa agaaacctcc catttgacaa aacaaccatc 1320
atggcagcat tcactgggaa tgcagaggga agaacatcag acatgagggc agaaatcata 1380
aggatgatgg aaggtgcaaa accagaagaa gtgtccttcc aggggcgggg agtcttcgag 1440
ctctcggacg aaaaggcaac gaacccgatc gtgccctctt ttgacatgag taatgaagga 1500
tcttatttct tcggagacaa tgcagaggag tacgacaatt aaggaaaaat accttgttt 1560
ctact 1565

<210> 6
<211> 1466
<212> DNA
<213> Influenza virus A/Singapore/1/57/ca

<400> 6
agcaaaagca ggagtgaaga tgaatccaaa tcaaaagata ataacaattg gctctgtctc 60
tctcaccatt gcaacagtat gcttcctcat gcagattgcc atcctggcaa ctactgtgac 120
attgcatttt aaacaacatg agtgcgactc ccccgcgagc aaccaagtaa tgccatgtga 180
accaataata atagaaagga acataacaga gatagtgtat ttgaataaca ccaccataga 240

gaaagagatt tgccccgaag tagtggaata cagaaattgg tcaaagccgc aatgtcaaat 300
tacaggattt gcacctttt ctaaggacaa ttcaatccgg ctttctgctg gtggggacat 360
ttgggtgacg agagaacctt atgtgtcatg cgatcctggc aagtgttatc aatttgcact 420
cgggcagggg accacactat acaacaaaca ttcaaatggc acaatacatg atagaatccc 480
tcatcgaacc ctattaatga atgagttggg tgttccattt catttaggaa ccaaacaagt 540
gtgtgtagca tggtccagct caagttgtca cgatggaaaa gcatggttgc atgtttgtgt 600
cactggggat gatagaaatg cgactgctag cttcatttat gacgggaggc ttgtggacag 660
tattggttca tggtctcaaa atatcctcag gacccaggag tcggaatgcg tttgtatcaa 720
tgggacttgc acagtagtaa tgactgatgg aagtgcatca ggaagagccg atactagaat 780
actattcatt aaagagggga aaattgtccg tattagccca ttgtcaggaa gtgctcagca 840
tatagaggag tgttcctgtt accctcgata tcctgacgtc agatgtatct gcagagacaa 900
ctggaaaggc tctaataggc ccgttataga cataaatatg gaagattata gcattgattc 960
cagttatgtg tgctcagggc ttgttggcga cacacccagg aacgacgaca gctctagcaa 1020
tagcaattgc agggatccta acaatgagag agggaatcca ggagtgaaag gctgggcctt 1080
tgacaatgga gatgatgtat ggatgggaag aacaatcaac aaagattcac gctcaggtta 1140
tgaaactttc aaagtcattg gtggttggtc cacacctaat tccaaatcgc aggtcaatag 1200
acaggtcata gttgacaaca ataattggtc tggttactct ggtattttct ctgttgaggg 1260
caaaagctgc atcaataggt gcttttatgt ggagttgata aggggaaggc cacaggagac 1320
tagagtatgg tggacctcaa acagtattgt tgtgttttgt ggcacttcag gtacttatgg 1380
aacaggctca tggcctgatg gggcgaacat caatttcatg cctatataag ctttcgcaat 1440
tttagaaaaa actccttgtt tctact 1466

<210> 7
<211> 1027
<212> DNA
<213> Influenza virus A/Singapore/1/57/ca

<400> 7
agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtatgttct 60
ctctatcgtc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt 120
tgctgggaag aacaccgatc ttgaggctct catggaatgg ctaaagacaa gaccaatcct 180
gtcacctctg actaagggga ttttgggatt tgtattcacg ctcaccgtgc ccagtgagcg 240
aggactgcag cgtagacgct ttgtccaaaa tgccctcaat gggaatgggg atccaaataa 300
catggacaga gcagttaaac tgtataaaaa gcttaagagg gagataacat tccatggggc 360
caaagaaata gcgctcagtt attctgctgg tgcacttgcc agttgtatgg gcctcatata 420
caacaggatg ggggctgtga ccactgaagt ggcctttggc ctggtatgtg caacctgtga 480
acagattgct gactcccacc ataggtctca taggcaaatg gtgacaacaa ccaatccact 540
aataagacat gagaacagaa tggttctggc cagcactaca gctaaggcta tggagcaaat 600
ggctggatcg agtgagcaag cagcagaggc catggaggtt gctagtcagg ccaggcaaat 660
ggtgcaggca atgagagcca ttgggactca tcctagctcc agtgctggtc taaaagatga 720
tcttcttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacgattcaa 780
gtgaccctct tgttgttgcc gcgagtatca ttgggatctt gcacttgata ttgtggattc 840
ttgatcgtct ttttttcaaa tgcatttatc gcttctttaa acacggtctg aaaagagggc 900
cttctacgga aggagtacca gagtctatga gggaagaata tcgaaaggaa cagcagagtg 960
ctgtggatgc tgacgatagt cattttgtca gcatagagct ggagtaaaaa actaccttgt 1020
ttctact 1027

<210> 8

<211> 890
<212> DNA
<213> Influenza virus A/Singapore/1/57/ca

<400> 8
agcaaaagca gggtgacaaa gacataatgg atcctaacac tgtgtcaagc tttcaggtag 60
attgcttcct ttggcatgtc cgcaaacaag ttgcagacca agaactaggt gatgccccat 120
tccttgatcg gcttcgccga gatcagaagt ccctaagggg aagaggcagc actctcggtc 180
tgaacatcga aacagccacc cgtgttggaa agcagatagt ggagaggatt ctgaaggaag 240
aatccgatga ggcacttaaa atgaccatgg cctccgcacc tgcttcgcga tacctaactg 300
acatgactat tgaggaaatg tcaagggact ggttcatgct aatgcccaag cagaaagtgt 360
caggccctct ttgtatcaga atggaccagg caatcatgga taagaacatc atattgaaag 420
cgaatttcag tgtgattttt gaccggctag agaccctaat attactaagg gctttcaccg 480
aagagggagc aattgttggc gaaatttcac cattgccttc tcttccagga catactaatg 540
aggatgtcaa aaatgcaatt ggggtcctca tcggaggact tgaatggaat gataacacag 600
ttcgagtctc taaaactcta cagagattcg cttggagaaa cagtaatgag aatgggagac 660
ctccactcac tccaaaacag aaacggaaaa tggcgagaac aattaggtca aaagttcgaa 720
gaaataagat ggctgattga agaagtgaga cacaaattga agataacaga gaatagtttt 780
gagcaaataa catttatgca agccttacag ctgctatttg aagtggaaca agagataaga 840
actttctcgt ttcagcttat ttaatgataa aaaacaccct tgtttctact 890

<210> 9
<211> 771
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 9
Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr
1 5 10 15

Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys
20 25 30

Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys
35 40 45

Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr
50 55 60

Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
65 70 75 80

Met Asn Asp Ala Gly Ser Asp Arg Val Met Val Ser Pro Leu Ala Val
85 90 95

Thr Trp Trp Asn Arg Asn Gly Pro Met Thr Ser Thr Val His Tyr Pro
100 105 110

```
Lys Ile Tyr Lys Thr Tyr Phe Glu Lys Val Glu Arg Leu Lys His Gly
        115                 120                 125

Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg
    130                 135                 140

Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln
145                 150                 155                 160

Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile
                165                 170                 175

Leu Thr Ser Glu Ser Gln Leu Thr Thr Thr Lys Glu Lys Lys Glu Glu
            180                 185                 190

Leu Gln Asp Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu
        195                 200                 205

Arg Glu Leu Val Arg Lys Thr Arg Phe Leu Pro Val Ala Gly Gly Thr
    210                 215                 220

Ser Ser Val Tyr Ile Glu Val Leu His Leu Thr Gln Gly Thr Cys Trp
225                 230                 235                 240

Glu Gln Met Tyr Thr Pro Gly Gly Glu Val Arg Asn Asp Asp Val Asp
                245                 250                 255

Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Ala Val
            260                 265                 270

Ser Ala Asp Pro Leu Ala Ser Leu Leu Glu Met Cys His Ser Thr Gln
        275                 280                 285

Ile Gly Gly Thr Arg Met Val Asp Ile Leu Arg Gln Asn Pro Thr Glu
    290                 295                 300

Glu Gln Ala Val Asp Ile Cys Lys Ala Ala Met Gly Leu Arg Ile Ser
305                 310                 315                 320

Ser Ser Phe Ser Phe Gly Gly Phe Thr Phe Lys Arg Thr Ser Gly Ser
                325                 330                 335

Ser Val Lys Ile Glu Glu Glu Val Leu Thr Gly Asn Leu Gln Thr Leu
            340                 345                 350

Lys Ile Arg Val His Glu Gly Tyr Glu Glu Phe Thr Met Val Gly Lys
        355                 360                 365
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Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Ile Gln Leu
    370                 375                 380

Ile Val Ser Gly Arg Asp Glu Gln Ser Ile Ala Glu Ala Ile Ile Val
385                 390                 395                 400

Ala Met Val Phe Ser Gln Glu Asp Cys Met Ile Lys Ala Val Arg Gly
                405                 410                 415

Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His
            420                 425                 430

Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Gln Asn
        435                 440                 445

Trp Gly Ile Glu His Ile Asp Asn Val Met Gly Met Ile Gly Val Leu
    450                 455                 460

Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Val Arg Val
465                 470                 475                 480

Ser Lys Met Gly Val Asp Glu Tyr Ser Ser Ala Glu Arg Val Val Val
                485                 490                 495

Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu
            500                 505                 510

Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr
        515                 520                 525

Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser
    530                 535                 540

Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val
545                 550                 555                 560

Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu
                565                 570                 575

Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr
            580                 585                 590

Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly
        595                 600                 605

Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala
    610                 615                 620
```

```
Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val
625                 630                 635                 640

Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Val Phe
                645                 650                 655

Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala
            660                 665                 670

Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ser Gly Val Glu Ser
        675                 680                 685

Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr
    690                 695                 700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu
705                 710                 715                 720

Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys
                725                 730                 735

Arg Lys Arg Asp Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys
            740                 745                 750

Arg Ile Arg Met Ala Ile Asn Xaa Cys Xaa Ile Val Xaa Lys Arg Pro
        755                 760                 765

Cys Phe Tyr
    770
```

<210> 10
<211> 757
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 10

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Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn
  1               5                  10                  15

Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His
             20                  25                  30

Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln
         35                  40                  45

Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala Pro
     50                  55                  60
```

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Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
 65                  70                  75                  80

Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu
                 85                  90                  95

Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Leu Glu Thr Met Glu
            100                 105                 110

Val Ile Gln Gln Thr Arg Val Asp Lys Leu Thr Gln Gly Arg Gln Thr
        115                 120                 125

Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala
    130                 135                 140

Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser
145                 150                 155                 160

Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Ile Glu Ser Met Asp Lys
                165                 170                 175

Glu Glu Met Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg
            180                 185                 190

Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys
        195                 200                 205

Lys Gln Arg Leu Asn Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu
    210                 215                 220

Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
225                 230                 235                 240

Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu
                245                 250                 255

Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro
            260                 265                 270

Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys
        275                 280                 285

Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly
    290                 295                 300

Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala
305                 310                 315                 320
```

Met Ile Thr Tyr Ile Thr Arg Asn Gln Pro Glu Trp Phe Arg Asn Val
325 330 335

Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly
340 345 350

Lys Gly Tyr Met Phe Glu Ser Lys Ser Met Lys Leu Arg Thr Gln Ile
355 360 365

Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser
370 375 380

Thr Arg Lys Lys Ile Glu Lys Ile Arg Pro Leu Leu Ile Asp Gly Thr
385 390 395 400

Val Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser
405 410 415

Thr Val Ile Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr
420 425 430

Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala
435 440 445

Leu Ile Val Asn Ala Pro Asn His Glu Gly Ile Gln Ala Gly Val Asp
450 455 460

Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys
465 470 475 480

Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe
485 490 495

Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe
500 505 510

Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr
515 520 525

Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala
530 535 540

Gln Met Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg
545 550 555 560

Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu
565 570 575

```
Lys Lys Leu Trp Glu Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser
            580                 585                 590

Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu
        595                 600                 605

Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu
    610                 615                 620

Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val
625                 630                 635                 640

Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu
                645                 650                 655

Tyr Asp Ala Val Ala Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg
            660                 665                 670

Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met
        675                 680                 685

Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser
    690                 695                 700

Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser
705                 710                 715                 720

Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys
                725                 730                 735

Lys Glu Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu
            740                 745                 750

Leu Arg Arg Gln Lys
        755
```

<210> 11
<211> 716
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 11

```
Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
  1               5                  10                  15

Ala Glu Arg Ala Met Lys Glu Tyr Gly Glu Asp Leu Lys Ile Glu Thr
```

```
            20                  25                  30

Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr
         35                  40                  45

Ser Asp Phe His Phe Ile Asn Glu Gln Gly Glu Ser Ile Ile Val Glu
     50                  55                  60

Leu Asp Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu
 65                  70                  75                  80

Gly Arg Asp Arg Thr Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn
                 85                  90                  95

Thr Thr Gly Ala Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
            100                 105                 110

Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His
        115                 120                 125

Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Lys Thr His
    130                 135                 140

Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp
145                 150                 155                 160

Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe
                165                 170                 175

Thr Ile Arg Gln Glu Met Ala Ser Arg Gly Leu Trp Asp Ser Phe Arg
            180                 185                 190

Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Arg Phe Glu Ile Thr
        195                 200                 205

Gly Thr Met Arg Arg Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Ser
    210                 215                 220

Cys Leu Glu Ile Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly
225                 230                 235                 240

Tyr Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Lys
                245                 250                 255

Ile Glu Pro Phe Leu Lys Thr Thr Pro Arg Pro Ile Arg Leu Pro Asp
            260                 265                 270

Gly Pro Pro Cys Ser Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu
```

275 280 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu
290 295 300

Tyr Asp Ala Ile Lys Cys Met Arg Thr Phe Phe Gly Trp Lys Glu Pro
305 310 315 320

Tyr Val Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Leu
325 330 335

Ser Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu
340 345 350

Lys Ile Pro Arg Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp
355 360 365

Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys
370 375 380

Arg Asp Ile Ser Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu
385 390 395 400

Arg Ser Leu Ser Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu
405 410 415

Leu Thr Asn Ser Ile Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val
420 425 430

Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala
435 440 445

Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr
450 455 460

Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe
465 470 475 480

Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg
485 490 495

Lys Thr Asn Leu Tyr Gly Phe Ile Val Lys Gly Arg Ser His Leu Arg
500 505 510

Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr
515 520 525

Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu

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    530                 535                 540

Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Val Ser Arg Pro
545                 550                 555                 560

Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys
                565                 570                 575

Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile
            580                 585                 590

Glu Ser Met Ile Glu Ala Gln Ser Ser Val Lys Glu Lys Asp Met Thr
        595                 600                 605

Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser
    610                 615                 620

Pro Lys Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu
625                 630                 635                 640

Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu
                645                 650                 655

Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Val Val Gln Ala Leu
            660                 665                 670

Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu
        675                 680                 685

Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala
    690                 695                 700

Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Arg
705                 710                 715


<210> 12
<211> 562
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 12
Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Asp
  1               5                  10                  15

Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Lys Val Asp
            20                  25                  30
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Thr Ile Leu Glu Gln Asn Val Thr Val Thr His Ala Lys Asp Ile Leu
         35                  40                  45

Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro
     50                  55                  60

Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro
 65                  70                  75                  80

Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu
                 85                  90                  95

Lys Glu Asn Pro Arg Asp Gly Leu Cys Tyr Pro Gly Ser Phe Asn Asp
            100                 105                 110

Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys
        115                 120                 125

Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly
    130                 135                 140

Gly Ser Arg Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn
145                 150                 155                 160

Met Val Trp Leu Thr Lys Lys Glu Ser Asn Tyr Pro Val Ala Lys Gly
                165                 170                 175

Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val
            180                 185                 190

His His Pro Asn Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val
        195                 200                 205

Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr
    210                 215                 220

Pro Asp Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met
225                 230                 235                 240

Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu
                245                 250                 255

Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys
            260                 265                 270

Arg Gly Asn Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys
        275                 280                 285
```

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Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro
    290                 295                 300

Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val
305                 310                 315                 320

Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Pro Arg Asn Val Pro Gln
                325                 330                 335

Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly
            340                 345                 350

Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn
        355                 360                 365

Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala
    370                 375                 380

Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Met Asn
385                 390                 395                 400

Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg
                405                 410                 415

Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp
            420                 425                 430

Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu
        435                 440                 445

Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met
    450                 455                 460

Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe
465                 470                 475                 480

Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr
                485                 490                 495

Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu
            500                 505                 510

Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Tyr Gln Ile Leu Ala
        515                 520                 525

Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala
    530                 535                 540
```

```
Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile
545                 550                 555                 560

Cys Ile
```

<210> 13
<211> 506
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 13

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Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
  1               5                  10                  15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met
             20                  25                  30

Ile Asp Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
         35                  40                  45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
     50                  55                  60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
 65                  70                  75                  80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
                 85                  90                  95

Tyr Lys Arg Val Asn Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp
            100                 105                 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp
        115                 120                 125

Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn
    130                 135                 140

Asp Thr Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145                 150                 155                 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
                165                 170                 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu
            180                 185                 190
```

Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
195 200 205

Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
210 215 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp
225 230 235 240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
245 250 255

Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
260 265 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Thr Ala Val Ala Ser Gly
275 280 285

Tyr Asp Phe Glu Lys Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
290 295 300

Lys Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu
305 310 315 320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys Asn Ser Ala
325 330 335

Ala Phe Glu Asp Leu Arg Val Ser Ser Phe Ile Arg Gly Thr Lys Val
340 345 350

Ile Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
355 360 365

Glu Asn Met Asp Thr Met Glu Ser Ser Thr Leu Glu Leu Arg Ser Arg
370 375 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg
385 390 395 400

Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg
405 410 415

Asn Leu Pro Phe Asp Lys Thr Thr Ile Met Ala Ala Phe Thr Gly Asn
420 425 430

Ala Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met
435 440 445

Glu Gly Ala Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe
450 455 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465 470 475 480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
485 490 495

Asp Asn Xaa Gly Lys Ile Pro Leu Phe Leu
500 505

<210> 14
<211> 469
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 14
Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Val Ser Leu Thr
1 5 10 15

Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala Ile Leu Ala Thr Thr
20 25 30

Val Thr Leu His Phe Lys Gln His Glu Cys Asp Ser Pro Ala Ser Asn
35 40 45

Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
50 55 60

Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Glu
65 70 75 80

Val Val Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
85 90 95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
100 105 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Gly Lys
115 120 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Tyr Asn Lys His
130 135 140

Ser Asn Gly Thr Ile His Asp Arg Ile Pro His Arg Thr Leu Leu Met

145 150 155 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Val
165 170 175

Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
180 185 190

Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Ile Tyr Asp
195 200 205

Gly Arg Leu Val Asp Ser Ile Gly Ser Trp Ser Gln Asn Ile Leu Arg
210 215 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val
225 230 235 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
245 250 255

Ile Lys Glu Gly Lys Ile Val Arg Ile Ser Pro Leu Ser Gly Ser Ala
260 265 270

Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asp Val Arg
275 280 285

Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp
290 295 300

Ile Asn Met Glu Asp Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly
305 310 315 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Asn Ser Asn
325 330 335

Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp
340 345 350

Ala Phe Asp Asn Gly Asp Asp Val Trp Met Gly Arg Thr Ile Asn Lys
355 360 365

Asp Ser Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Ser
370 375 380

Thr Pro Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn
385 390 395 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser

405 410 415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln
420 425 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
435 440 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
450 455 460

Asn Phe Met Pro Ile
465

<210> 15
<211> 252
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 15
Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro
1 5 10 15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
20 25 30

Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr
35 40 45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
50 55 60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65 70 75 80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Arg Ala
85 90 95

Val Lys Leu Tyr Lys Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
100 105 110

Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
115 120 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
130 135 140

```
Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser His His Arg
145                 150                 155                 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
                165                 170                 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
            180                 185                 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
        195                 200                 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser
    210                 215                 220

Ser Ser Ala Gly Leu Lys Asp Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225                 230                 235                 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
                245                 250
```

<210> 16
<211> 97
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 16

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Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly
  1               5                  10                  15

Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Ser Ile
            20                  25                  30

Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp Arg Leu Phe Phe
        35                  40                  45

Lys Cys Ile Tyr Arg Phe Phe Lys His Gly Leu Lys Arg Gly Pro Ser
    50                  55                  60

Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln
65                  70                  75                  80

Gln Ser Ala Val Asp Ala Asp Asp Ser His Phe Val Ser Ile Glu Leu
                85                  90                  95

Glu
```

<210> 17
<211> 237
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 17

```
Met Asp Pro Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp
  1               5                  10                  15

His Val Arg Lys Gln Val Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe
             20                  25                  30

Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser
         35                  40                  45

Thr Leu Gly Leu Asn Ile Glu Thr Ala Thr Arg Val Gly Lys Gln Ile
     50                  55                  60

Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr
 65                  70                  75                  80

Met Ala Ser Ala Pro Ala Ser Arg Tyr Leu Thr Asp Met Thr Ile Glu
                 85                  90                  95

Glu Met Ser Arg Asp Trp Phe Met Leu Met Pro Lys Gln Lys Val Ser
            100                 105                 110

Gly Pro Leu Cys Ile Arg Met Asp Gln Ala Ile Met Asp Lys Asn Ile
        115                 120                 125

Ile Leu Lys Ala Asn Phe Ser Val Ile Phe Asp Arg Leu Glu Thr Leu
    130                 135                 140

Ile Leu Leu Arg Ala Phe Thr Glu Glu Gly Ala Ile Val Gly Glu Ile
145                 150                 155                 160

Ser Pro Leu Pro Ser Leu Pro Gly His Thr Asn Glu Asp Val Lys Asn
                165                 170                 175

Ala Ile Gly Val Leu Ile Gly Gly Leu Glu Trp Asn Asp Asn Thr Val
            180                 185                 190

Arg Val Ser Lys Thr Leu Gln Arg Phe Ala Trp Arg Asn Ser Asn Glu
        195                 200                 205

Asn Gly Arg Pro Pro Leu Thr Pro Lys Gln Lys Arg Lys Met Ala Arg
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    210                 215                 220

Thr Ile Arg Ser Lys Val Arg Arg Asn Lys Met Ala Asp
225                 230                 235


<210> 18
<211> 121
<212> PRT
<213> Influenza virus A/Singapore/1/57/ca

<400> 18
Met Asp Pro Asn Thr Val Ser Ser Phe Gln Asp Ile Leu Met Arg Met
  1               5                  10                  15

Ser Lys Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile
             20                  25                  30

Thr Gln Phe Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Thr
         35                  40                  45

Val Met Arg Met Gly Asp Leu His Ser Leu Gln Asn Arg Asn Gly Lys
     50                  55                  60

Trp Arg Glu Gln Leu Gly Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile
 65                  70                  75                  80

Glu Glu Val Arg His Lys Leu Lys Ile Thr Glu Asn Ser Phe Glu Gln
                 85                  90                  95

Ile Thr Phe Met Gln Ala Leu Gln Leu Leu Phe Glu Val Glu Gln Glu
            100                 105                 110

Ile Arg Thr Phe Ser Phe Gln Leu Ile
        115                 120


<210> 19
<211> 2396
<212> DNA
<213> Influenza B/Vienna/1/99/ca

<400> 19
agcagaagcg gagcgttttc aagatgacat tggctaaaat tgaattgtta aaacaactgt 60
taagggacaa tgaagccaaa acagtattga aacaaacaac agtagatcaa tataacataa 120
taagaaaatt caatacatca agaattgaaa agaacccttc attaaggatg aagtgggcaa 180
tgtgttctaa ttttcccttg gctttgacca agggtgacat ggcaaacaga atccccttgg 240
aatacaaggg aatacaactt aaaacaaatg ctgaagacat aggaaccaaa ggccaaatgt 300
```

gctcaatagc agcagttacc tggtggaata catatggacc aataggagat actgaaggtt 360
tcgaaaaagt ctacgaaagc ttttttctca gaaagatgag acttgacaat gccacttggg 420
gccgaataac ttttggccca gttgaaagag taagaaaaag ggtactgcta aaccctctca 480
ccaaggaaat gcctccagat gaagcaagta atgtgataat ggaaatattg ttccctaagg 540
aagcaggaat accaagagaa tctacttgga tacataggga actgataaaa gaaaaaagag 600
aaaaattgaa aggaacgatg ataactccca ttgtactggc atacatgctt gagagggaat 660
tggttgccag gagaaggttc ctgccggtag caggagcaac atcagctgag ttcatagaaa 720
tgctacactg cttacaaggt gaaaattgga gacaaatata tcacccggga gggaataaac 780
taactgaatc taggtctcaa tcgatgattg tggcttgtag aaagataatc agaagatcaa 840
tagtcgcatc aaacccattg gagctagctg tagaaattgc aaacaagact gtaatagata 900
ctgaaccttt aaaatcatgt ctgacagcca tagacggagg tgatgtcgcc tgtgacataa 960
taagggctgc attaggacta aagatcagac aaagacaaag atttggacga cttgaactaa 1020
agagaatatc aggaagagga ttcaaaaatg atgaagaaat attaatcggg aacggaacaa 1080
tacagaagat tggaatatgg gacggagaag aggagttcca tgtaagatgt ggtgaatgca 1140
ggggaatatt aaaaaagagc aaaatgagaa tggaaaaact actaataaat tcagctaaaa 1200
aggaagacat gaaagattta ataatcttgt gcatggtatt ttctcaagac actaggatgt 1260
tccaaggagt gaggggagaa ataaattttc ttaatagagc aggccaactt ttatctccaa 1320
tgtatcaact ccaaagatat tttttgaata gaagtaatga tctctttgat caatgggggt 1380
atgaggaatc acccaaagca agtgagctac atgggataaa tgaattaatg aatgcatctg 1440
actacacttt gaaaggggtt gtagtaacaa aaaatgtgat tgatgatttt agttctactg 1500
aaacagaaaa agtatctata acaaaaaatc ttagtttaat aaaaaggact ggggaagtca 1560
taatgggagc caatgacgta agtgaattag aatcacaagc tcagctaatg ataacatatg 1620
atacacctaa gatgtgggag atgggaacaa ccaaagaact ggtgcaaaac acctatcaat 1680
gggtgctgaa aaatttggta acactgaagg ctcagtttct tctaggaaaa gaagacatgt 1740
tccaatggga tgcatttgaa gcatttgaaa gcataatccc ccagaagatg gctggccaat 1800
acagtggatt tgcaagagca gtgctcaaac aaatgagaga ccaagaggtc atgaaaactg 1860
accagttcat aaagttgttg ccctttttgtt tctcaccacc aaagttaagg agcaatgggg 1920
agccttatca gttcttgagg cttgtattga agggaggagg agaaaatttc atcgaagtaa 1980
ggaaagggtc tcctctattc tcttacaatc cacaaacaga agtcctaact atatgcggca 2040
gaatgatgtc attaaaaggg aaaattgaag atgaagaaag gaatagatca atggggaatg 2100
cagtgttggc gggttttctt gttagtggca agtatgaccc agatcttgga gatttcaaaa 2160
ctattgaaga gcttgaaaag ctaaaaccgg gggagaaagc aaacatctta ctttatcaag 2220
gaaagcccgt taaagtagtt aaaaggaaaa gatatagtgc tttatccaat gacatttcac 2280
aaggaattaa gagacaaaga atgacagttg agtccatggg gtgggccttg agctaatata 2340
aatttatcca ttaattcaat gaatgcaatt gagtgaaaaa tgctcgtgtt tctcat 2396

<210> 20
<211> 2369
<212> DNA
<213> Influenza B/Vienna/1/99/ca

<400> 20
agcagaagcg gagcctttaa gatgaatata aatccttatt ttctcttcat agatgtaccc 60
atacaggcag caatttcaac aacattccca tacaccggtg ttccccctta ttcccatgga 120
acgggaacag gccacacaat agacaccgtg atcagaacac atgagtactc gaacaaagga 180
aaacagtatg tttctgacat cacaggatgt acaatggtag atccaacaaa tggaccatta 240
cccgaagaca atgagccaag tgcctatgca caattagatt gcgttctgga ggctttggat 300
agaatggatg aggaacatcc aggtctgttt caagcagcct cacagaatgc catggaggca 360

ctaatggtca caactgtaga caaattaacc caggggagac agactttcga ttggacagta 420
tgcagaaatc agcctgctgc aacggcacta aacacaacaa taacctcctt taggttgaat 480
gatttgaatg gagctgacaa gggtggattg gtacccttt gccaagatat cattgattca 540
ttagacaagc ctgaaatgac tttcttctca gtaaagaata taaagaaaaa attccctgct 600
aaaaacagaa agggtttcct cataaagaga ataccaatga aagtaaaaga caggatatcc 660
agagtggaat acatcaaaag agcattgtca ttaaacacaa tgacaaaaga tgctgaaagg 720
ggcaaactaa aaagaagagc gattgcaacc gctggaatac aaatcagagg gtttgtatta 780
gtagttgaaa acttggctaa aaatatctgt gaaaatctag aacaaagtgg tttgcccgta 840
ggtggaaatg aaaagaaggc caaactgtca aatgcagtgg ccaaaatgct cagtaactgc 900
ccaccaggag ggatcagcat gacagtaaca ggagacaata ctaaatggaa tgaatgctta 960
aatccaagag tctttttggc tatgactgaa agaataacca gagacagccc aatttggttc 1020
cgggattttt gtagtatagc accggtcttg ttctccaata aaatagccag attgggaaaa 1080
ggatttatga taacaagtaa aacaaaaaga ctgaaggctc aaatacctg tcctgatctg 1140
tttagcatac cattagaaag atataatgaa gaaacaaggg caaaattaaa aaagctgaaa 1200
ccattcttca atgaagaagg aacggcatct ttgtcgcctg gaatgatgat gggaatgttt 1260
aatatgctat ctaccgtgtt gggagtagca gcactaggta tcaaaaacat tggaaacaag 1320
gaatacttat gggatggact gcaatcttct gatgattttg ctctgtttgt taatgcaaaa 1380
gatgaagaga catgtatgga aggaataaac gatttttacc gaacatgtaa attattggga 1440
ataaacatga gcaaaaagaa aagttactgt aacgaaactg gaatgtttga atttacaagc 1500
atgttctata gagatggatt tgtatctaac tttgcaatgg aaattccttc atttggagtt 1560
gctggagtaa atgaatcagc agatatggca ataggaatga caataataaa gaacaatatg 1620
attaacaatg ggatgggtcc agcaacagca caaacagcca tacaattgtt catagctgat 1680
tataggtaca cctacaaatg ccacagagga gattccaaag tggaaggaaa aagaatgaaa 1740
attataaagg agctatggga aaacactaaa ggaagagatg gtctgttagt ggcagatggt 1800
gggcccaaca tttacaattt gagaaactta catatcccag aaatagtatt gaagtacaat 1860
ctaatggacc ctgaatacaa agggcggtta cttcaccctc aaaatccctt tgtaggacat 1920
ttgtctattg aaggcatcaa agaagcagat ataaccccag cacatggtcc tgtgaagaaa 1980
atggattatg atgcagtgtc tggaactcat agttggagaa ccaaaaggaa cagatctata 2040
ctaaatactg atcagaggaa catgattctt gaggaacaat gctacgctaa atgttgcaac 2100
ctttttgagg cctgttttaa cagtgcatca tacaggaaac cagtagggca gcacagcatg 2160
cttgaggcta tggcccatag attaagaatg gatgcacgac tggattatga atcaggaaga 2220
atgtcaaagg atgattttga gaaagcaatg gctcaccttg gtgagattgg gtacacataa 2280
gctccgaaga tgtccatggg gttattggtc atcattggat acatgtgata aacaaatgat 2340
taaaatgaaa aaaggctcgt gtttctact 2369

<210> 21
<211> 2305
<212> DNA
<213> Influenza B/Vienna/1/99/ca

<400> 21
agcagaagcg gtgcgtttga tttgtcataa tggatacttt tattacaaga aacttccaga 60
ctacaataat acaaaaggcc aaaaacacaa tggcagaatt tagtgaagat cctgaattac 120
aaccagcaat gctattcaac atctgcgtcc atctagaggt ttgctatgta ataagtgaca 180
tgaattttct tgacgaagaa ggaaaagcat atacagcatt agaaggacaa gggaaagaac 240
aaaatttgag accacaatat gaagtaattg agggaatgcc aagaaccata gcatggatgg 300
tccaaagatc cttagctcaa gagcatggaa tagagactcc caagtatctg gctgatttgt 360

ttgattataa aaccaagaga tttatagaag ttggaataac aaaaggattg gctgatgatt 420
acttttggaa aaagaaagaa aagctgggaa atagcatgga actgatgata ttcagctaca 480
atcaagacta ttcgttaagt aatgaatcct cattggatga ggaagggaaa gggagagtgc 540
taagcagact cacagaactt caggctgaat taagtctgaa aaacctatgg caagttctca 600
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<210> 22
<211> 1882
<212> DNA
<213> Influenza B/Vienna/1/99/ca

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<210> 23
<211> 1844
<212> DNA
<213> Influenza B/Vienna/1/99/ca

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gatgtcggga ggaaaaccca aaagaaacag accccgacag agataaagaa gagcgtctac 300
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cattcacaga tgaatgatgt ctgtttccaa agatcaaagg cactaaaaag agttggactt 720
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<210> 24
<211> 1557
<212> DNA
<213> Influenza B/Vienna/1/99/ca

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cagaaataac tgcaccaaca atgccattgg attgtgcaaa cgcatcaaat gttcaggctg 240
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<210> 25
<211> 1190
<212> DNA
<213> Influenza B/Vienna/1/99/ca

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<210> 26
<211> 1097
<212> DNA
<213> Influenza B/Vienna/1/99/ca

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<210> 27
<211> 770
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 27

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Glu Ala Lys Thr Val Leu Lys Gln Thr Thr Val Asp Gln Tyr Asn Ile
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Ile Arg Lys Phe Asn Thr Ser Arg Ile Glu Lys Asn Pro Ser Leu Arg
35 40 45

Met Lys Trp Ala Met Cys Ser Asn Phe Pro Leu Ala Leu Thr Lys Gly
50 55 60

Asp Met Ala Asn Arg Ile Pro Leu Glu Tyr Lys Gly Ile Gln Leu Lys
65 70 75 80

Thr Asn Ala Glu Asp Ile Gly Thr Lys Gly Gln Met Cys Ser Ile Ala
85 90 95

Ala Val Thr Trp Trp Asn Thr Tyr Gly Pro Ile Gly Asp Thr Glu Gly
100 105 110

Phe Glu Lys Val Tyr Glu Ser Phe Phe Leu Arg Lys Met Arg Leu Asp
115 120 125

Asn Ala Thr Trp Gly Arg Ile Thr Phe Gly Pro Val Glu Arg Val Arg
130 135 140

Lys Arg Val Leu Leu Asn Pro Leu Thr Lys Glu Met Pro Pro Asp Glu
145 150 155 160

Ala Ser Asn Val Ile Met Glu Ile Leu Phe Pro Lys Glu Ala Gly Ile
165 170 175

Pro Arg Glu Ser Thr Trp Ile His Arg Glu Leu Ile Lys Glu Lys Arg
180 185 190

Glu Lys Leu Lys Gly Thr Met Ile Thr Pro Ile Val Leu Ala Tyr Met

195 200 205

Leu Glu Arg Glu Leu Val Ala Arg Arg Arg Phe Leu Pro Val Ala Gly
210 215 220

Ala Thr Ser Ala Glu Phe Ile Glu Met Leu His Cys Leu Gln Gly Glu
225 230 235 240

Asn Trp Arg Gln Ile Tyr His Pro Gly Gly Asn Lys Leu Thr Glu Ser
245 250 255

Arg Ser Gln Ser Met Ile Val Ala Cys Arg Lys Ile Ile Arg Arg Ser
260 265 270

Ile Val Ala Ser Asn Pro Leu Glu Leu Ala Val Glu Ile Ala Asn Lys
275 280 285

Thr Val Ile Asp Thr Glu Pro Leu Lys Ser Cys Leu Thr Ala Ile Asp
290 295 300

Gly Gly Asp Val Ala Cys Asp Ile Ile Arg Ala Ala Leu Gly Leu Lys
305 310 315 320

Ile Arg Gln Arg Gln Arg Phe Gly Arg Leu Glu Leu Lys Arg Ile Ser
325 330 335

Gly Arg Gly Phe Lys Asn Asp Glu Glu Ile Leu Ile Gly Asn Gly Thr
340 345 350

Ile Gln Lys Ile Gly Ile Trp Asp Gly Glu Glu Glu Phe His Val Arg
355 360 365

Cys Gly Glu Cys Arg Gly Ile Leu Lys Lys Ser Lys Met Arg Met Glu
370 375 380

Lys Leu Leu Ile Asn Ser Ala Lys Lys Glu Asp Met Lys Asp Leu Ile
385 390 395 400

Ile Leu Cys Met Val Phe Ser Gln Asp Thr Arg Met Phe Gln Gly Val
405 410 415

Arg Gly Glu Ile Asn Phe Leu Asn Arg Ala Gly Gln Leu Leu Ser Pro
420 425 430

Met Tyr Gln Leu Gln Arg Tyr Phe Leu Asn Arg Ser Asn Asp Leu Phe
435 440 445

Asp Gln Trp Gly Tyr Glu Glu Ser Pro Lys Ala Ser Glu Leu His Gly

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450                 455                 460

Ile Asn Glu Leu Met Asn Ala Ser Asp Tyr Thr Leu Lys Gly Val Val
465                 470                 475                 480

Val Thr Lys Asn Val Ile Asp Asp Phe Ser Ser Thr Glu Thr Glu Lys
                485                 490                 495

Val Ser Ile Thr Lys Asn Leu Ser Leu Ile Lys Arg Thr Gly Glu Val
            500                 505                 510

Ile Met Gly Ala Asn Asp Val Ser Glu Leu Glu Ser Gln Ala Gln Leu
        515                 520                 525

Met Ile Thr Tyr Asp Thr Pro Lys Met Trp Glu Met Gly Thr Thr Lys
    530                 535                 540

Glu Leu Val Gln Asn Thr Tyr Gln Trp Val Leu Lys Asn Leu Val Thr
545                 550                 555                 560

Leu Lys Ala Gln Phe Leu Leu Gly Lys Glu Asp Met Phe Gln Trp Asp
                565                 570                 575

Ala Phe Glu Ala Phe Glu Ser Ile Ile Pro Gln Lys Met Ala Gly Gln
            580                 585                 590

Tyr Ser Gly Phe Ala Arg Ala Val Leu Lys Gln Met Arg Asp Gln Glu
        595                 600                 605

Val Met Lys Thr Asp Gln Phe Ile Lys Leu Leu Pro Phe Cys Phe Ser
    610                 615                 620

Pro Pro Lys Leu Arg Ser Asn Gly Glu Pro Tyr Gln Phe Leu Arg Leu
625                 630                 635                 640

Val Leu Lys Gly Gly Gly Glu Asn Phe Ile Glu Val Arg Lys Gly Ser
                645                 650                 655

Pro Leu Phe Ser Tyr Asn Pro Gln Thr Glu Val Leu Thr Ile Cys Gly
            660                 665                 670

Arg Met Met Ser Leu Lys Gly Lys Ile Glu Asp Glu Glu Arg Asn Arg
        675                 680                 685

Ser Met Gly Asn Ala Val Leu Ala Gly Phe Leu Val Ser Gly Lys Tyr
    690                 695                 700

Asp Pro Asp Leu Gly Asp Phe Lys Thr Ile Glu Glu Leu Glu Lys Leu
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705 710 715 720

Lys Pro Gly Glu Lys Ala Asn Ile Leu Leu Tyr Gln Gly Lys Pro Val
725 730 735

Lys Val Val Lys Arg Lys Arg Tyr Ser Ala Leu Ser Asn Asp Ile Ser
740 745 750

Gln Gly Ile Lys Arg Gln Arg Met Thr Val Glu Ser Met Gly Trp Ala
755 760 765

Leu Ser
770

<210> 28
<211> 752
<212> PRT
<213> Influenza B/Vienna/1/99/ca

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Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Val Pro Pro Tyr Ser His
20 25 30

Gly Thr Gly Thr Gly His Thr Ile Asp Thr Val Ile Arg Thr His Glu
35 40 45

Tyr Ser Asn Lys Gly Lys Gln Tyr Val Ser Asp Ile Thr Gly Cys Thr
50 55 60

Met Val Asp Pro Thr Asn Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
65 70 75 80

Ala Tyr Ala Gln Leu Asp Cys Val Leu Glu Ala Leu Asp Arg Met Asp
85 90 95

Glu Glu His Pro Gly Leu Phe Gln Ala Ala Ser Gln Asn Ala Met Glu
100 105 110

Ala Leu Met Val Thr Thr Val Asp Lys Leu Thr Gln Gly Arg Gln Thr
115 120 125

Phe Asp Trp Thr Val Cys Arg Asn Gln Pro Ala Ala Thr Ala Leu Asn
130 135 140

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Thr Thr Ile Thr Ser Phe Arg Leu Asn Asp Leu Asn Gly Ala Asp Lys
145                 150                 155                 160

Gly Gly Leu Val Pro Phe Cys Gln Asp Ile Ile Asp Ser Leu Asp Lys
                165                 170                 175

Pro Glu Met Thr Phe Phe Ser Val Lys Asn Ile Lys Lys Lys Phe Pro
            180                 185                 190

Ala Lys Asn Arg Lys Gly Phe Leu Ile Lys Arg Ile Pro Met Lys Val
        195                 200                 205

Lys Asp Arg Ile Ser Arg Val Glu Tyr Ile Lys Arg Ala Leu Ser Leu
    210                 215                 220

Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
225                 230                 235                 240

Ile Ala Thr Ala Gly Ile Gln Ile Arg Gly Phe Val Leu Val Val Glu
                245                 250                 255

Asn Leu Ala Lys Asn Ile Cys Glu Asn Leu Glu Gln Ser Gly Leu Pro
            260                 265                 270

Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ser Asn Ala Val Ala Lys
        275                 280                 285

Met Leu Ser Asn Cys Pro Pro Gly Gly Ile Ser Met Thr Val Thr Gly
    290                 295                 300

Asp Asn Thr Lys Trp Asn Glu Cys Leu Asn Pro Arg Val Phe Leu Ala
305                 310                 315                 320

Met Thr Glu Arg Ile Thr Arg Asp Ser Pro Ile Trp Phe Arg Asp Phe
                325                 330                 335

Cys Ser Ile Ala Pro Val Leu Phe Ser Asn Lys Ile Ala Arg Leu Gly
            340                 345                 350

Lys Gly Phe Met Ile Thr Ser Lys Thr Lys Arg Leu Lys Ala Gln Ile
        355                 360                 365

Pro Cys Pro Asp Leu Phe Ser Ile Pro Leu Glu Arg Tyr Asn Glu Glu
    370                 375                 380

Thr Arg Ala Lys Leu Lys Lys Leu Lys Pro Phe Phe Asn Glu Glu Gly
385                 390                 395                 400
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Thr Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu
                405                 410                 415

Ser Thr Val Leu Gly Val Ala Ala Leu Gly Ile Lys Asn Ile Gly Asn
            420                 425                 430

Lys Glu Tyr Leu Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala Leu
        435                 440                 445

Phe Val Asn Ala Lys Asp Glu Glu Thr Cys Met Glu Gly Ile Asn Asp
    450                 455                 460

Phe Tyr Arg Thr Cys Lys Leu Leu Gly Ile Asn Met Ser Lys Lys Lys
465                 470                 475                 480

Ser Tyr Cys Asn Glu Thr Gly Met Phe Glu Phe Thr Ser Met Phe Tyr
                485                 490                 495

Arg Asp Gly Phe Val Ser Asn Phe Ala Met Glu Ile Pro Ser Phe Gly
            500                 505                 510

Val Ala Gly Val Asn Glu Ser Ala Asp Met Ala Ile Gly Met Thr Ile
        515                 520                 525

Ile Lys Asn Asn Met Ile Asn Asn Gly Met Gly Pro Ala Thr Ala Gln
    530                 535                 540

Thr Ala Ile Gln Leu Phe Ile Ala Asp Tyr Arg Tyr Thr Tyr Lys Cys
545                 550                 555                 560

His Arg Gly Asp Ser Lys Val Glu Gly Lys Arg Met Lys Ile Ile Lys
                565                 570                 575

Glu Leu Trp Glu Asn Thr Lys Gly Arg Asp Gly Leu Leu Val Ala Asp
            580                 585                 590

Gly Gly Pro Asn Ile Tyr Asn Leu Arg Asn Leu His Ile Pro Glu Ile
        595                 600                 605

Val Leu Lys Tyr Asn Leu Met Asp Pro Glu Tyr Lys Gly Arg Leu Leu
    610                 615                 620

His Pro Gln Asn Pro Phe Val Gly His Leu Ser Ile Glu Gly Ile Lys
625                 630                 635                 640

Glu Ala Asp Ile Thr Pro Ala His Gly Pro Val Lys Lys Met Asp Tyr
                645                 650                 655
```

```
Asp Ala Val Ser Gly Thr His Ser Trp Arg Thr Lys Arg Asn Arg Ser
            660                 665                 670

Ile Leu Asn Thr Asp Gln Arg Asn Met Ile Leu Glu Glu Gln Cys Tyr
        675                 680                 685

Ala Lys Cys Cys Asn Leu Phe Glu Ala Cys Phe Asn Ser Ala Ser Tyr
    690                 695                 700

Arg Lys Pro Val Gly Gln His Ser Met Leu Glu Ala Met Ala His Arg
705                 710                 715                 720

Leu Arg Met Asp Ala Arg Leu Asp Tyr Glu Ser Gly Arg Met Ser Lys
                725                 730                 735

Asp Asp Phe Glu Lys Ala Met Ala His Leu Gly Glu Ile Gly Tyr Thr
            740                 745                 750
```

<210> 29
<211> 726
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 29

```
Met Asp Thr Phe Ile Thr Arg Asn Phe Gln Thr Thr Ile Ile Gln Lys
  1               5                  10                  15

Ala Lys Asn Thr Met Ala Glu Phe Ser Glu Asp Pro Glu Leu Gln Pro
             20                  25                  30

Ala Met Leu Phe Asn Ile Cys Val His Leu Glu Val Cys Tyr Val Ile
         35                  40                  45

Ser Asp Met Asn Phe Leu Asp Glu Glu Gly Lys Ala Tyr Thr Ala Leu
     50                  55                  60

Glu Gly Gln Gly Lys Glu Gln Asn Leu Arg Pro Gln Tyr Glu Val Ile
 65                  70                  75                  80

Glu Gly Met Pro Arg Thr Ile Ala Trp Met Val Gln Arg Ser Leu Ala
                 85                  90                  95

Gln Glu His Gly Ile Glu Thr Pro Lys Tyr Leu Ala Asp Leu Phe Asp
            100                 105                 110
```

```
Tyr Lys Thr Lys Arg Phe Ile Glu Val Gly Ile Thr Lys Gly Leu Ala
        115                 120                 125

Asp Asp Tyr Phe Trp Lys Lys Lys Glu Lys Leu Gly Asn Ser Met Glu
    130                 135                 140

Leu Met Ile Phe Ser Tyr Asn Gln Asp Tyr Ser Leu Ser Asn Glu Ser
145                 150                 155                 160

Ser Leu Asp Glu Glu Gly Lys Gly Arg Val Leu Ser Arg Leu Thr Glu
                165                 170                 175

Leu Gln Ala Glu Leu Ser Leu Lys Asn Leu Trp Gln Val Leu Ile Gly
            180                 185                 190

Glu Glu Asp Val Glu Lys Gly Ile Asp Phe Lys Leu Gly Gln Thr Ile
        195                 200                 205

Ser Arg Leu Arg Asp Ile Ser Val Pro Ala Gly Phe Ser Asn Phe Glu
    210                 215                 220

Gly Met Arg Ser Tyr Ile Asp Asn Ile Asp Pro Lys Gly Ala Ile Glu
225                 230                 235                 240

Arg Asn Leu Ala Arg Met Ser Pro Leu Val Ser Val Thr Pro Lys Lys
                245                 250                 255

Leu Lys Trp Glu Asp Leu Arg Pro Ile Gly Pro His Ile Tyr Asn His
            260                 265                 270

Glu Leu Pro Glu Val Pro Tyr Asn Ala Phe Leu Leu Met Ser Asp Glu
        275                 280                 285

Leu Gly Leu Ala Asn Met Thr Glu Gly Lys Ser Lys Lys Pro Lys Thr
    290                 295                 300

Leu Ala Lys Glu Cys Leu Glu Lys Tyr Ser Thr Leu Arg Asp Gln Thr
305                 310                 315                 320

Asp Pro Ile Leu Ile Met Lys Ser Glu Lys Ala Asn Glu Asn Phe Leu
                325                 330                 335

Trp Lys Leu Trp Arg Asp Cys Val Asn Thr Ile Ser Asn Glu Glu Met
            340                 345                 350

Ser Asn Glu Leu Gln Lys Thr Asn Tyr Ala Lys Trp Ala Thr Gly Asp
        355                 360                 365
```

```
Gly Leu Thr Tyr Gln Lys Ile Met Lys Glu Val Ala Ile Asp Asp Glu
    370                 375                 380

Thr Met Cys Gln Glu Glu Pro Lys Ile Pro Asn Lys Cys Arg Val Ala
385                 390                 395                 400

Ala Trp Val Gln Thr Glu Met Asn Leu Leu Ser Thr Leu Thr Ser Lys
                405                 410                 415

Lys Ala Leu Asp Leu Pro Glu Ile Gly Pro Asp Val Ala Pro Val Glu
            420                 425                 430

His Val Gly Ser Glu Arg Arg Lys Tyr Phe Val Asn Glu Ile Asn Tyr
        435                 440                 445

Cys Lys Ala Ser Thr Val Met Met Lys Tyr Val Leu Phe His Thr Ser
    450                 455                 460

Leu Leu Asn Glu Ser Asn Ala Ser Met Gly Lys Tyr Lys Val Ile Pro
465                 470                 475                 480

Ile Thr Asn Arg Val Val Asn Glu Lys Gly Glu Ser Phe Asp Met Leu
                485                 490                 495

Tyr Gly Leu Ala Val Lys Gly Gln Ser His Leu Arg Gly Asp Thr Asp
            500                 505                 510

Val Val Thr Val Val Thr Phe Glu Phe Ser Ser Thr Asp Pro Arg Val
        515                 520                 525

Asp Ser Gly Lys Trp Pro Lys Tyr Thr Val Phe Arg Ile Gly Ser Leu
    530                 535                 540

Phe Val Ser Gly Arg Glu Lys Ser Val Tyr Leu Tyr Cys Arg Val Asn
545                 550                 555                 560

Gly Thr Asn Lys Ile Gln Met Lys Trp Gly Met Glu Ala Arg Arg Cys
                565                 570                 575

Leu Leu Gln Ser Met Gln Gln Met Glu Ala Ile Val Glu Gln Glu Ser
            580                 585                 590

Ser Ile Gln Gly Tyr Asp Met Thr Lys Ala Cys Phe Lys Gly Asp Arg
        595                 600                 605

Val Asn Ser Pro Lys Thr Phe Ser Ile Gly Thr Gln Glu Gly Lys Leu
    610                 615                 620
```

```
Val Lys Gly Ser Phe Gly Lys Ala Leu Arg Val Ile Phe Thr Lys Cys
625                 630                 635                 640

Leu Met His Tyr Val Phe Gly Asn Ala Gln Leu Glu Gly Phe Ser Ala
                645                 650                 655

Glu Ser Arg Arg Leu Leu Leu Leu Ile Gln Ala Leu Lys Asp Arg Lys
            660                 665                 670

Gly Pro Trp Val Phe Asp Leu Glu Gly Met Tyr Ser Gly Ile Glu Glu
        675                 680                 685

Cys Ile Ser Asn Asn Pro Trp Val Ile Gln Ser Ala Tyr Trp Phe Asn
    690                 695                 700

Glu Trp Leu Gly Phe Glu Lys Glu Gly Ser Lys Val Leu Glu Ser Val
705                 710                 715                 720

Asp Glu Ile Met Asp Glu
                725
```

<210> 30
<211> 584
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 30

```
Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
  1               5                  10                  15

Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
             20                  25                  30

Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Ala Ile Pro Leu Thr
         35                  40                  45

Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys Thr
     50                  55                  60

Arg Gly Lys Leu Cys Pro Thr Cys Leu Asn Cys Thr Asp Leu Asp Val
 65                  70                  75                  80

Ala Leu Gly Arg Pro Met Cys Val Gly Ile Thr Pro Ser Ala Lys Ala
                 85                  90                  95

Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
```

100 105 110

Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly
115 120 125

Tyr Glu Lys Ile Arg Leu Ser Thr Gln Asn Val Ile Asn Thr Glu Lys
130 135 140

Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn
145 150 155 160

Ala Thr Ser Lys Ser Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
165 170 175

Arg Asp Asn Asn Lys Thr Ala Thr Asn Pro Leu Thr Val Glu Val Pro
180 185 190

His Ile Cys Thr Lys Glu Glu Asp Gln Ile Thr Val Trp Gly Phe His
195 200 205

Ser Asp Asn Lys Thr Gln Met Lys Asn Leu Tyr Gly Asp Ser Asn Pro
210 215 220

Gln Lys Phe Thr Ser Ser Ala Asn Gly Ile Thr Thr His Tyr Val Ser
225 230 235 240

Gln Ile Gly Gly Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln
245 250 255

Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Pro Gly Lys Thr
260 265 270

Gly Thr Ile Val Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys Val Trp
275 280 285

Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile
290 295 300

Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser
305 310 315 320

Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro
325 330 335

Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg
340 345 350

Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala

355 360 365

Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly
370 375 380

Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys
385 390 395 400

Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu
405 410 415

Ser Glu Leu Glu Val Asn Asn Leu Gln Arg Leu Ser Gly Ala Met Asp
420 425 430

Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu
435 440 445

Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser
450 455 460

Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu
465 470 475 480

Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn
485 490 495

Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg
500 505 510

Ile Ala Ala Gly Thr Phe Asn Ala Glu Glu Phe Ser Leu Pro Thr Phe
515 520 525

Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp
530 535 540

Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala
545 550 555 560

Val Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Ile Ser Arg Asp
565 570 575

Asn Val Ser Cys Ser Ile Cys Leu
580

<210> 31
<211> 560
<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 31

```
Met Ser Asn Met Asp Ile Asp Gly Ile Asn Thr Gly Thr Ile Asp Lys
  1               5                  10                  15

Thr Pro Glu Glu Ile Thr Phe Gly Thr Ser Gly Thr Thr Arg Pro Ile
             20                  25                  30

Ile Arg Pro Ala Thr Leu Ala Pro Pro Ser Asn Lys Arg Thr Arg Asn
         35                  40                  45

Pro Ser Pro Glu Arg Ala Thr Thr Ser Ser Glu Ala Asp Val Gly Arg
     50                  55                  60

Lys Thr Gln Lys Lys Gln Thr Pro Thr Glu Ile Lys Lys Ser Val Tyr
 65                  70                  75                  80

Asn Met Val Val Lys Leu Gly Glu Phe Tyr Asn Gln Met Met Val Lys
                 85                  90                  95

Ala Gly Leu Asn Asp Asp Met Glu Arg Asn Leu Ile Gln Asn Ala His
            100                 105                 110

Ala Val Glu Arg Ile Leu Leu Ala Ala Thr Asp Asp Lys Lys Thr Glu
        115                 120                 125

Phe Gln Lys Lys Lys Asn Thr Arg Asp Val Lys Glu Gly Lys Glu Glu
    130                 135                 140

Ile Asp His Asn Lys Thr Gly Gly Thr Phe Tyr Lys Met Val Arg Asp
145                 150                 155                 160

Asp Lys Thr Ile Tyr Phe Ser Pro Ile Arg Ile Thr Phe Leu Lys Glu
                165                 170                 175

Glu Val Lys Thr Met Tyr Lys Thr Thr Met Gly Ser Asp Gly Phe Ser
            180                 185                 190

Gly Leu Asn His Ile Met Ile Gly His Ser Gln Met Asn Asp Val Cys
        195                 200                 205

Phe Gln Arg Ser Lys Ala Leu Lys Arg Val Gly Leu Asp Pro Ser Leu
    210                 215                 220

Ile Ser Thr Phe Ala Gly Ser Thr Ile Pro Arg Arg Ser Gly Ala Thr
225                 230                 235                 240
```

```
Gly Val Ala Ile Lys Gly Gly Gly Thr Leu Val Ala Glu Ala Ile Arg
                245                 250                 255

Phe Ile Gly Arg Ala Met Ala Asp Arg Gly Leu Leu Arg Asp Ile Lys
            260                 265                 270

Ala Lys Thr Ala Tyr Glu Lys Ile Leu Leu Asn Leu Lys Asn Lys Cys
        275                 280                 285

Ser Ala Pro Gln Gln Lys Ala Leu Val Asp Gln Val Ile Gly Ser Arg
    290                 295                 300

Asn Pro Gly Ile Ala Asp Ile Glu Asp Leu Thr Leu Leu Ala Arg Ser
305                 310                 315                 320

Met Val Val Val Arg Pro Ser Val Ala Ser Lys Val Val Leu Pro Ile
                325                 330                 335

Ser Ile Tyr Ala Lys Ile Pro Gln Leu Gly Phe Asn Val Glu Glu Tyr
            340                 345                 350

Ser Met Val Gly Tyr Glu Ala Met Ala Leu Tyr Asn Met Ala Thr Pro
        355                 360                 365

Val Ser Ile Leu Arg Met Gly Asp Asp Ala Lys Asp Lys Ser Gln Leu
    370                 375                 380

Phe Phe Met Ser Cys Phe Gly Ala Ala Tyr Glu Asp Leu Arg Val Leu
385                 390                 395                 400

Ser Ala Leu Thr Gly Thr Glu Phe Lys Pro Arg Ser Ala Leu Lys Cys
                405                 410                 415

Lys Gly Phe His Val Pro Ala Lys Glu Gln Val Glu Gly Met Gly Ala
            420                 425                 430

Ala Leu Met Ser Ile Lys Leu Gln Phe Trp Ala Pro Met Thr Arg Ser
        435                 440                 445

Gly Gly Asn Glu Val Gly Gly Asp Gly Gly Ser Gly Gln Ile Ser Cys
    450                 455                 460

Ser Pro Val Phe Ala Val Glu Arg Pro Ile Ala Leu Ser Lys Gln Ala
465                 470                 475                 480

Val Arg Arg Met Leu Ser Met Asn Ile Glu Gly Arg Asp Ala Asp Val
                485                 490                 495
```

```
Lys Gly Asn Leu Leu Lys Met Met Asn Asp Ser Met Ala Lys Lys Thr
            500                 505                 510

Ser Gly Asn Ala Phe Ile Gly Lys Lys Met Phe Gln Ile Ser Asp Lys
        515                 520                 525

Asn Lys Thr Asn Pro Val Glu Ile Pro Ile Lys Gln Thr Ile Pro Asn
    530                 535                 540

Phe Phe Phe Gly Arg Asp Thr Ala Glu Asp Tyr Asp Asp Leu Asp Tyr
545                 550                 555                 560
```

<210> 32
<211> 100
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 32

```
Met Asn Asn Ala Thr Phe Asn Tyr Thr Asn Val Asn Pro Ile Pro His
  1               5                  10                  15

Ile Arg Gly Ser Val Ile Ile Thr Ile Cys Val Ser Phe Thr Val Ile
             20                  25                  30

Leu Ile Ile Phe Gly Tyr Ile Ala Lys Ile Phe Thr Asn Arg Asn Asn
         35                  40                  45

Cys Thr Asn Asn Ala Ile Gly Leu Cys Lys Arg Ile Lys Cys Ser Gly
     50                  55                  60

Cys Glu Pro Phe Cys Asn Lys Arg Gly Asp Thr Ser Ser Pro Arg Thr
 65                  70                  75                  80

Gly Val Asp Ile Pro Ala Phe Ile Leu Pro Gly Leu Asn Leu Ser Glu
                 85                  90                  95

Ser Thr Pro Asn
            100
```

<210> 33
<211> 466
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 33

```
Met Leu Pro Ser Thr Ile Gln Thr Leu Thr Leu Phe Leu Thr Ser Gly
 1               5                  10                  15

Gly Val Leu Leu Ser Leu Tyr Val Ser Ala Ser Leu Ser Tyr Leu Leu
            20                  25                  30

Tyr Ser Asp Ile Leu Leu Lys Phe Ser Pro Thr Glu Ile Thr Ala Pro
        35                  40                  45

Thr Met Pro Leu Asp Cys Ala Asn Ala Ser Asn Val Gln Ala Val Asn
    50                  55                  60

Arg Ser Ala Thr Lys Gly Val Thr Leu Leu Leu Pro Glu Pro Glu Trp
65                  70                  75                  80

Thr Tyr Pro Arg Leu Ser Cys Pro Gly Ser Thr Phe Gln Lys Ala Leu
                85                  90                  95

Leu Ile Ser Pro His Arg Phe Gly Glu Thr Lys Gly Asn Ser Ala Pro
            100                 105                 110

Leu Ile Ile Arg Glu Pro Phe Ile Ala Cys Gly Pro Lys Glu Cys Lys
        115                 120                 125

His Phe Ala Leu Thr His Tyr Ala Ala Gln Pro Gly Gly Tyr Tyr Asn
    130                 135                 140

Gly Thr Arg Glu Asp Arg Asn Lys Leu Arg His Leu Ile Ser Val Lys
145                 150                 155                 160

Leu Gly Lys Ile Pro Thr Val Glu Asn Ser Ile Phe His Met Ala Ala
                165                 170                 175

Trp Ser Gly Ser Ala Cys His Asp Gly Lys Glu Trp Thr Tyr Ile Gly
            180                 185                 190

Val Asp Gly Pro Asp Ser Asn Ala Leu Leu Lys Ile Lys Tyr Gly Glu
        195                 200                 205

Ala Tyr Thr Asp Thr Tyr His Ser Tyr Ala Asn Asn Ile Leu Arg Thr
    210                 215                 220

Gln Glu Ser Ala Cys Asn Cys Ile Gly Gly Asn Cys Tyr Leu Met Ile
225                 230                 235                 240

Thr Asp Gly Ser Ala Ser Gly Ile Ser Glu Cys Arg Phe Leu Lys Ile
```

245 250 255

Gln Glu Gly Arg Ile Ile Lys Glu Ile Phe Pro Thr Gly Arg Val Glu
260 265 270

His Thr Glu Glu Cys Thr Cys Gly Phe Ala Ser Asn Lys Thr Ile Glu
275 280 285

Cys Ala Cys Arg Asp Asn Ser Tyr Thr Ala Lys Arg Pro Phe Val Lys
290 295 300

Leu Asn Val Glu Thr Asp Thr Ala Glu Ile Arg Leu Met Cys Thr Glu
305 310 315 320

Thr Tyr Leu Asp Thr Pro Arg Pro Asp Asp Gly Ser Ile Thr Gly Pro
325 330 335

Cys Glu Ser Asn Gly Asp Lys Gly Ser Gly Gly Ile Lys Gly Gly Phe
340 345 350

Val His Gln Arg Met Ala Ser Lys Thr Gly Arg Trp Tyr Ser Arg Thr
355 360 365

Met Ser Lys Thr Lys Arg Met Gly Met Gly Leu Tyr Val Lys Tyr Asp
370 375 380

Gly Asp Pro Trp Thr Asp Ser Asp Ala Leu Ala Leu Ser Gly Val Met
385 390 395 400

Val Ser Met Glu Glu Pro Gly Trp Tyr Ser Phe Gly Phe Glu Ile Lys
405 410 415

Asp Lys Lys Cys Asp Val Pro Cys Ile Gly Ile Glu Met Val His Asp
420 425 430

Gly Gly Lys Glu Thr Trp His Ser Ala Ala Thr Ala Ile Tyr Cys Leu
435 440 445

Met Gly Ser Gly Gln Leu Leu Trp Asp Thr Val Thr Gly Val Asn Met
450 455 460

Ala Leu
465

<210> 34
<211> 248
<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 34

```
Met Ser Leu Phe Gly Asp Thr Ile Ala Tyr Leu Leu Ser Leu Thr Glu
  1               5                  10                  15

Asp Gly Glu Gly Lys Ala Glu Leu Ala Glu Lys Leu His Cys Trp Phe
             20                  25                  30

Gly Gly Lys Glu Phe Asp Leu Asp Ser Ala Leu Glu Trp Ile Lys Asn
         35                  40                  45

Lys Arg Cys Leu Thr Asp Ile Gln Lys Ala Leu Ile Gly Ala Ser Ile
     50                  55                  60

Cys Phe Leu Lys Pro Lys Asp Gln Glu Arg Lys Arg Arg Phe Ile Thr
 65                  70                  75                  80

Glu Pro Leu Ser Gly Met Gly Thr Thr Ala Thr Lys Lys Lys Gly Leu
                 85                  90                  95

Ile Leu Ala Glu Arg Lys Met Arg Arg Cys Val Ser Phe His Glu Ala
            100                 105                 110

Phe Glu Ile Ala Glu Gly His Glu Ser Ser Ala Leu Leu Tyr Cys Leu
        115                 120                 125

Met Val Met Tyr Leu Asn Pro Gly Asn Tyr Ser Met Gln Val Lys Leu
    130                 135                 140

Gly Thr Leu Cys Ala Leu Cys Glu Lys Gln Ala Ser His Ser His Arg
145                 150                 155                 160

Ala His Ser Arg Ala Ala Arg Ser Ser Val Pro Gly Val Arg Arg Glu
                165                 170                 175

Met Gln Met Val Ser Ala Met Asn Thr Ala Lys Thr Met Asn Gly Met
            180                 185                 190

Gly Lys Gly Glu Asp Val Gln Lys Leu Ala Glu Glu Leu Gln Ser Asn
        195                 200                 205

Ile Gly Val Leu Arg Ser Leu Gly Ala Ser Gln Lys Asn Gly Glu Gly
    210                 215                 220

Ile Ala Lys Asp Val Met Glu Val Leu Lys Gln Ser Ser Met Gly Asn
225                 230                 235                 240
```

Ser Ala Leu Val Lys Lys Tyr Leu
245

<210> 35
<211> 109
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 35
Met Leu Glu Pro Phe Gln Ile Leu Ser Ile Cys Ser Phe Ile Leu Ser
1 5 10 15

Ala Leu His Phe Val Ala Trp Thr Ile Gly His Leu Asn Gln Ile Lys
20 25 30

Arg Gly Val Asn Met Lys Ile Arg Ile Lys Ser Pro Asn Lys Glu Thr
35 40 45

Ile Asn Arg Glu Val Ser Ile Leu Arg His Ser Tyr Gln Lys Glu Ile
50 55 60

Gln Ala Lys Glu Thr Met Lys Glu Val Leu Ser Asp Asn Met Glu Val
65 70 75 80

Leu Gly Asp His Ile Val Ile Glu Gly Leu Ser Ala Glu Glu Ile Ile
85 90 95

Lys Met Gly Glu Thr Val Leu Glu Ile Glu Glu Leu His
100 105

<210> 36
<211> 281
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 36
Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Val Gly Pro Gly Ala
1 5 10 15

Thr Asn Ala Thr Ile Asn Phe Glu Thr Gly Ile Leu Glu Cys Tyr Glu
20 25 30

Arg Leu Ser Trp Gln Arg Ala Leu Asp Tyr Pro Gly Gln Asp Arg Leu
35 40 45

Asn Arg Leu Lys Arg Lys Leu Glu Ser Arg Ile Lys Thr His Asn Lys

```
     50                  55                  60

Ser Glu Pro Glu Ser Lys Arg Met Ser Leu Glu Glu Arg Lys Ala Ile
 65                  70                  75                  80

Gly Val Lys Met Met Lys Val Leu Leu Phe Met Asn Pro Ser Ala Gly
                 85                  90                  95

Ile Glu Gly Phe Glu Pro Tyr Tyr Met Lys Ser Ser Ser Asn Ser Asn
            100                 105                 110

Cys Pro Lys Tyr Asn Trp Thr Asp Tyr Pro Ser Thr Pro Gly Arg Cys
        115                 120                 125

Leu Asp Asp Ile Glu Glu Glu Pro Glu Asp Val Asp Gly Pro Thr Glu
    130                 135                 140

Ile Val Leu Arg Asp Met Asn Asn Lys Asp Ala Arg Gln Lys Ile Lys
145                 150                 155                 160

Glu Glu Val Asn Thr Gln Lys Glu Gly Lys Phe Arg Leu Thr Ile Lys
                165                 170                 175

Arg Asp Ile Arg Asn Val Leu Ser Leu Arg Val Leu Val Asn Gly Thr
            180                 185                 190

Phe Leu Lys His Pro Asn Gly Tyr Lys Ser Leu Ser Thr Leu His Arg
        195                 200                 205

Leu Asn Ala Tyr Asp Gln Ser Gly Arg Leu Val Ala Lys Leu Val Ala
    210                 215                 220

Thr Asp Asp Leu Thr Val Glu Asp Glu Glu Asp Gly His Arg Ile Leu
225                 230                 235                 240

Asn Ser Leu Phe Glu Arg Leu Asn Glu Gly His Ser Lys Pro Ile Arg
                245                 250                 255

Ala Ala Glu Thr Ala Val Gly Val Leu Ser Gln Phe Gly Gln Glu His
            260                 265                 270

Arg Leu Ser Pro Glu Glu Gly Asp Asn
        275                 280
```

<210> 37
<211> 122
<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 37

```
Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Trp Arg Met Lys Lys
  1               5                  10                  15

Met Ala Ile Gly Ser Ser Thr His Ser Ser Ser Val Leu Met Lys Asp
             20                  25                  30

Ile Gln Ser Gln Phe Glu Gln Leu Lys Leu Arg Trp Glu Ser Tyr Pro
         35                  40                  45

Asn Leu Val Lys Ser Thr Asp Tyr His Gln Lys Arg Glu Thr Ile Lys
     50                  55                  60

Leu Val Thr Glu Glu Leu Tyr Leu Leu Ser Lys Arg Ile Asp Asp Asn
 65                  70                  75                  80

Ile Leu Phe His Lys Thr Val Ile Ala Asn Ser Ser Ile Ile Ala Asp
                 85                  90                  95

Met Val Val Ser Leu Ser Leu Leu Glu Thr Leu Tyr Glu Met Lys Asp
            100                 105                 110

Val Val Glu Val Tyr Ser Arg Gln Cys Leu
        115                 120
```